

**“INTEGRATED NETWORK PHARMACOLOGY, TRANSCRIPTOMIC VALIDATION,
AND MOLECULAR DOCKING ANALYSIS OF ANTI-INFLAMMATORY
MECHANISMS OF COLUMBIN”**

**A Thesis submitted
In partial fulfillment of the requirements
for the degree of the**

**MASTER OF TECHNOLOGY
In
INDUSTRIAL BIOTECHNOLOGY**

**by
ANANYA SINGH
24/IBT/01
Under the Supervision of
Prof. YASHA HASIJA
Head of Department,
Department of Biotechnology,
Delhi Technological University, Delhi**



**Department of Biotechnology
DELHI TECHNOLOGICAL UNIVERSITY
(Formerly Delhi College of Engineering)
Shahbad Daultapur, Main Bawana Road, Delhi-110042. India
May, 2026**



DELHI TECHNOLOGICAL UNIVERSITY

(Formerly Delhi College of Engineering)

Shahbad Daulatpur, Main Bawana Road, Delhi-42

CANDIDATE'S DECLARATION

I **Ananya Singh (24/IBT/01)** hereby certify that the work which is being presented in the thesis entitled “**Integrated Network Pharmacology, Transcriptomic Validation, And Molecular Docking Analysis of Anti-Inflammatory Mechanisms Of Columbin**” in partial fulfillment of the requirements for the award of the Degree of Doctor of Philosophy, submitted in the Department of Biotechnology, Delhi Technological University is an authentic record of my own work carried out during the period from January 2026 to May 2026 under the supervision of Prof. Yasha Hasija.

The matter presented in the thesis has not been submitted by me for the award of any other degree of this or any other Institute.

Candidate's Signature



DELHI TECHNOLOGICAL UNIVERSITY

(Formerly Delhi College of Engineering)

Shahbad Daulatpur, Main Bawana Road, Delhi-42

CERTIFICATE BY THE SUPERVISOR(s)

Certified that Ananya Singh (24/IBT/01) has carried out their search work presented in this thesis entitled “**Integrated Network Pharmacology, Transcriptomic Validation, And Molecular Docking Analysis Anti-Inflammatory Mechanisms Of Columbin**” for the award of **Master of Technology** from Department of Information Technology, Delhi Technological University, Delhi, under my supervision. The thesis embodies results of original work, and studies are carried out by the student herself and the contents of the thesis do not form the basis for the award of any other degree to the candidate or to anybody else from this or any other University/Institution.

Prof. Yasha Hasija

Supervisor, Head of Department

Department of Biotechnology

Delhi Technological University

Prof. Yasha Hasija

Head of Department

Department of Biotechnology

Delhi Technological University

Date:

INTEGRATED NETWORK PHARMACOLOGY, TRANSCRIPTOMIC VALIDATION, AND MOLECULAR DOCKING ANALYSIS ANTI-INFLAMMATORY MECHANISMS OF COLUMBIN

ABSTRACT

The intricacy of chronic inflammation's interrelated signaling pathways has led to the perception that multi-target treatment tactics are more successful than single-target approaches, emphasising the necessity of system-level research. Columbin, a furanoditerpenoid lactone discovered from the *Tinospora* species, has been shown to have anti-inflammation properties; however, the underlying molecular mechanisms remain unknown. This study used an integrated computational approach to thoroughly investigate columbin's multi-target anti-inflammatory properties. Four computational techniques were used to suggest potential targets, which were then confirmed against genes linked with inflammation using GeneCards, DisGeNET, and transcriptome profiling (GSE55235). Protein-protein interaction (PPI) analysis indicated eight hub genes (ESR1, JAK1, JAK2, MAPK1, STAT3, HSP90AA1, PIK3R1, and PTPN11) that contribute to significant inflammatory signaling. Molecular docking demonstrated favorable binding affinities. Molecular docking yielded favorable binding affinities (ΔG : -6.9 to -8.6 kcal/mol) and protocol validation using co-crystal ligand redocking (RMSD < 2 Å). External validation with an independent rheumatoid arthritis dataset (GSE55457) revealed differential expression of hub genes (2 of 8 significant, FDR <0.05) with diagnostic potential (5 of 8 genes, AUC >0.70). Immune infiltration research indicated strong relationships between hub gene expression and immune cell types, notably neutrophils and macrophages. ADMET profiling revealed favorable oral bioavailability with anticipated hepatotoxicity, requiring experimental confirmation. These findings provide the first comprehensive characterisation of columbin's multi-target anti-inflammatory mechanism, as well as a computational framework that may be used to phytochemical drug development.

KEYWORDS: ADMET Analysis, Columbin, Inflammation, Molecular docking, Molecular mechanism, Network pharmacology

ACKNOWLEDGEMENT

I would like to express my sincere gratitude to my supervisor, Prof. Yasha Hasija, for his constant guidance, valuable insights, encouragement, and support throughout the course of this research work. Her expertise, motivation, and constructive suggestions greatly contributed to the successful completion of this thesis. I am deeply thankful for the opportunity to work under his supervision and learn from his extensive knowledge and research experience.

I would also like to extend my heartfelt appreciation to the Ph.D. scholars and research students of the department for their guidance, technical assistance, and continuous support during various stages of this work. Their discussions and suggestions were immensely helpful in improving both the technical and analytical aspects of the study.

I sincerely thank the faculty members and staff of the Department of Biotechnology for providing the necessary academic environment, facilities, and resources required for carrying out this research successfully.

A special note of thanks goes to my classmates and friends for their encouragement, companionship, and support throughout this journey. Finally, I express my deepest gratitude to my family for their unconditional love, patience, understanding, and unwavering support throughout my academic journey. Their encouragement and belief in me have always been my greatest strength and motivation. This achievement would not have been possible without their continuous support and sacrifices.

ANANYA SINGH

24/IBT/01

TABLE OF CONTENTS

Candidate's Declaration.....	(ii)
Certificate by the Supervisor.....	(iii)
Abstract.....	(iv)
Acknowledgement.....	(v)
Table of Contents.....	(vi–viii)
List of Figures.....	(ix–x)
List of Tables.....	(xi)
List of Abbreviations.....	(xii–xiii)
Chapter 1 Introduction.....	(1-2)
Chapter 2 Literature Review.....	(3-28)
2.1 Inflammation: Biological Basis and Clinical Significance.....	3
2.2 The Molecular Mechanisms Underlying the Inflammatory Response.....	4
2.2.1 Inflammasomes.....	4
2.2.2 Pro-inflammatory Cytokines.....	4
2.2.3 Chemokines and Other Inflammatory Mediators.....	5
2.2.4 Role of Macrophages and Monocytes.....	6
2.2.5 Role of Neutrophils.....	6
2.2.6 Role of T Cells.....	7
2.3 Key Inflammatory Signaling Pathways in Chronic Inflammation.....	7
2.3.1 NF- κ B Signaling Pathway.....	8
2.3.2 MAPK Signaling Pathway.....	8
2.3.3 JAK-STAT Signaling Pathway.....	9
2.3.4 COX and LOX Pathways: Eicosanoid Signaling.....	10
2.4 Current Therapies and Limitations of Anti-inflammatory Medications.....	10
2.4.1 NSAIDs and Cyclooxygenase (COX) Inhibition.....	10
2.4.2 Corticosteroids.....	11
2.4.3 Biologics and Targeted Therapies.....	12
2.4.4 JAK Inhibitors and the Problem of Single-Target Therapy.....	13
2.4.5 Emerging Multi-Target Strategies.....	14
2.5 Medicinal Plants and Phytochemicals as Anti-inflammatory Drugs.....	14

2.5.1	Importance of Herbal Medicine.....	15
2.5.2	Secondary Metabolites: Anti-inflammatory Properties.....	15
2.5.3	Major Classes (Polyphenols, Alkaloids, Terpenoids).....	15
2.5.4	Multi-target Nature of Phytochemicals.....	16
2.6	Columbin: Sources, Chemistry and Pharmacological Profile.....	16
2.6.1	Botanical and Chemical Classification.....	17
2.6.2	Structure and Class.....	18
2.6.3	Pharmacological Activities.....	18
2.6.4	Evidence on COX Inhibition and Inflammatory Modulation.....	18
2.7	Network Pharmacology in Drug Discovery.....	19
2.7.1	Concept and Principles of Network Pharmacology	20
2.7.2	Multi-target Therapeutic Approaches.....	20
2.7.3	Application in Herbal Medicine and Natural Products.....	20
2.7.4	Identification of Compound–Target–Pathway Interactions	20
2.8	Application of Molecular Docking in Phytochemical Research.....	22
2.8.1	Principles of Molecular Docking.....	23
2.8.2	Protein–Ligand Interaction Analysis and Target Binding Validation.....	23
2.8.3	Importance and Applications in Phytochemical Research.....	23
2.9	Transcriptomic Validation and Bioinformatics Approaches in Target Discovery.....	25
2.9.1	Gene Expression Datasets and GEO.....	25
2.9.2	Validation of Predicted Therapeutic Targets.....	26
2.10	Research Gap and Rationale of the Present Study.....	27
Chapter 3	Materials and Methods.....	(29-34)
3.1	Identifying Columbin and Inflammation-related Targets.....	32
3.2	Venn Diagram and Construction of PPI Network.....	32
3.3	GO and KEGG Enrichment Analysis.....	32
3.4	Ligand and Protein Preparation.....	32
3.5	Molecular Docking.....	33
3.6	Immune Cell Infiltration Analysis.....	33
3.7	External Transcriptomics Validation.....	33
3.7.1	Gene Expression Analysis.....	33

3.7.2 ROC Curve Analysis.....	33
Chapter 4 Results and Discussion.....	(34-44)
4.1 Identification of Columbin and Disease Targets.....	34
4.2 PPI Network and Hub Gene Identification.....	34
4.3 Functional Enrichment Analysis.....	34
4.4 Molecular Docking Analysis.....	36
4.5 Immune Cell Infiltration Analysis.....	38
4.6 Bioinformatics Validation.....	39
4.6.1 Gene Expression Analysis.....	39
4.6.2 ROC Curve Analysis.....	40
4.7 ADMET Analysis.....	42
Chapter 5 Conclusion and Future Perspectives.....	(45-46)
Chapter 6 References.....	(47-64)
Plagiarism Verification.....	(65)

LIST OF FIGURES

Figure No.	Figure
Fig. 1	Flow chart for a network-based pharmacology study
Fig. 2	Network building and gene identification. (A) A volcano map of differentially expressed genes from GSE55235. (B) A Venn diagram of overlapping targets between columbin and inflammation-related genes.
Fig. 3	(A) PPI network with overlapping targets; (B) CytoHubba-identified hub gene network
Fig. 4	(A) Gene Ontology (GO) enrichment analysis reveals Biological Process (BP), Cellular Component (CC), and Molecular Function (MF). (B) KEGG pathway enrichment analysis identifies overlapping targets
Fig. 5	3D visualization of the docked ligand within the active site of the target protein, showing the binding pocket interactions and ligand orientation obtained from molecular docking studies – A-ESR1, B-JAK2, C-PIK3CA-PIK3R1, D-STAT3, E-HSP90AA1, F-JAK1, G-MAPK1 and H-PTPN11
Fig. 6	CIBERSORT-based heatmap showing the relative proportions of immune cell infiltration across samples
Fig. 7	(A) Boxplot showing differential gene expression in the GEO dataset between disease and control samples. (B) Heatmap illustrating the expression patterns of differentially expressed genes across samples in the GEO dataset
Fig. 8	Receiver operating characteristic (ROC) curve depicting the diagnostic performance of key genes with corresponding AUC values
Fig. 9	(A) A boiled-egg plot of columbin predicts gastrointestinal absorption and blood-brain barrier permeability (based on WLOGP and TPSA). Columbin is shown as a blue point. (B) The columbin bioavailability radar map

	depicts some of the main drug-likeness properties of columbin, including lipophilicity, size, polarity, solubility, flexibility, and saturation
--	---

LIST OF TABLES

Table No.	Table
Table 1	Molecular docking results of columbin and native ligand with core targets
Table 2	AUC values from ROC analysis representing the diagnostic performance of core targets
Table 3	ADMET properties of columbin predicted using in silico tools, including absorption, distribution, metabolism, excretion, toxicity, and drug-likeness parameters

LIST OF ABBREVIATIONS

Abbreviation	Full Form
ADMET	Absorption, Distribution, Metabolism, Excretion and Toxicity
COX	Cyclooxygenase
DGE	Differential Gene Expression
ERK	Extracellular Signal-Regulated Kinase
FDR	False Discovery Rate
GBM	Glioblastoma
GEO	Gene Expression Omnibus
GO	Gene Ontology
IBD	Inflammatory Bowel Disease
IFN	Interferon
IL	Interleukin
JAK	Janus Kinase
JNK	c-Jun N-terminal kinase
KEGG	Kyoto Encyclopedia of Genes and Genomes
LOX	Lipoxygenase
LPS	Lipopolysaccharide
MAPK	Mitogen-Activated Protein Kinase
NF	Nuclear Factor
NF- κ B	Nuclear Factor kappa-light-chain-enhancer of activated B cells
NO	Nitric Oxide
NSAIDs	Non-Steroidal Anti-Inflammatory Drugs
PPI	Protein-Protein Interaction
PRR	Pattern Recognition Receptor
RA	Rheumatoid Arthritis
RMSD	Root Mean Square Deviation

RNA	Ribonucleic Acid
ROS	Reactive Oxygen Species
SPR	Surface Plasmon Resonance
STAT	Signal Transducer and Activator of Transcription
TCM	Traditional Chinese Medicine
TLR	Toll-Like Receptor
TNF	Tumor Necrosis Factor
WGCNA	Weighted Gene Co-expression Network Analysis
iNOS	Inducible Nitric Oxide Synthase

CHAPTER - 1

INTRODUCTION

Inflammation is a key biological activity required for host defense and tissue healing. Persistent or improperly regulated inflammatory responses are closely associated with the development of several severe health conditions affecting different organ systems, including autoimmune joint disorders, chronic intestinal inflammation, cardiovascular complications caused by plaque buildup, and progressive neurological degeneration. Together, these long-term inflammatory conditions contribute to nearly 60% of mortality across the globe (1,2). Conventional anti-inflammation treatments, such as NSAIDs, steroids, and biologic agents that block pro-inflammatory mediators like TNF- α and IL-6, have greatly improved the management of acute inflammatory conditions and autoimmune diseases. However, each class has major limits that prevent prolonged usage. NSAIDs primarily block cyclooxygenase (COX) and give symptomatic relief, although they are linked with gastrointestinal damage, renal impairment, and cardiovascular problems, particularly with long-term usage (3). Corticosteroids, while extremely effective, have significant long-term toxicity that includes osteoporosis, gastrointestinal ulcers, metabolic changes, immunosuppression, and avascular necrosis (4). Biologic treatments targeting TNF- α , IL-6, or JAK-STAT signalling show benefit in 40-60% of patients with RA and IBD. However, their high cost and immunosuppressive effects restrict accessibility and long-term safety. Importantly, these medicines often target specific inflammatory mediators, which may be inadequate in illnesses defined by network-level dysregulation and route redundancy (5). The acute inflammatory response is critical for host defense and consists of a synchronized network of cytokines, kinases, transcription factors, and immune effector cells. When this coordination is broken by environmental triggers, genetic predisposition, or chronic pathogenic stressors, the ensuing dysregulation includes several interwoven signaling pathways rather than discrete molecular abnormalities (6,7). This single-target strategy frequently fails to address the complexity of inflammatory networks, resulting in unsatisfactory response rates and treatment resistance.

The increased interest in herbal medicine has prompted a change in pharmacological research towards isolating bioactive ingredients obtained from medicinal plants (8). Columbin, a key constituent of the Menispermaceae family, which comprises *Tinospora* sp. and *Jateorhiza palmata*, was found as an anti-inflammatory drug in several exploratory studies (9–11). In vitro studies show that it inhibits cyclooxygenase (COX) enzymes more effectively against COX 1 (about 64% inhibition at 100 μ M) than COX 2 (approximately 19% at 100 μ M) (12). However, the molecular targets and network-level processes that underpin columbin's anti-inflammatory effect remain poorly understood.

This study uses network pharmacology, molecular docking, as well as external transcriptomic validation to thoroughly identify and describe columbin's molecular targets, as well as their interactions with important inflammatory signaling networks. This systems-level approach gives a thorough grasp of both the interaction landscape and its biological importance. These findings are the first comprehensive characterisation of columbin's multi-target anti-inflammatory mechanisms, and they offer a computational paradigm that may be used to other phytochemical lead optimization initiatives.

Objectives

- To explore the potential biomolecular interactions of columbin associated with inflammation-related pathways through the application of network pharmacology and computational bioinformatics approaches.
- To identify the binding interactions between columbin and major inflammatory proteins using molecular docking and assess the biological significance of these potential targets using external transcriptomic data.

CHAPTER - 2

LITERATURE REVIEW

2.1 Inflammation: Biological Basis and Clinical Significance

Inflammation is required for host defense, tissue repair, and homeostasis restoration. Acute inflammation generally subsides quickly, is transient and is restricted (13) and is responsible for eliminating pathogens and repairing tissues. On the other hand it becomes chronic inflammation when the inflammatory stimuli is present for a long time or if there are no resolution mechanisms. This, in turn, can result in continuous damage to tissues, organs and disease development (14).

Inflammation, which is important for the elimination of pathogens and damaged cells, is mediated by recognition of patterns receptors from innate immunity, cytokines, inflammasomes, macrophages, neutrophils, and so on; the acute inflammatory response is distinguished by rapid cytokine release and immune cell infiltration.

Sustained, mild inflammatory activity over prolonged periods has been strongly associated with the process of aging and is considered an important contributor in the development of several diseases, such as metabolic abnormalities, heart-related conditions, malignancies, autoimmune joint complications, chronic intestinal disorders, and progressive neurological degeneration (15). Chronic inflammation can result in tissue remodeling, an increase in oxidative stress, genomic instability and the development of disease (16).

Macrophages and neutrophils are key role-players of inflammatory processes. Neutrophils contribute to antimicrobial action by phagocytosis and NETs, whereas macrophages are responsible for pathogen clearance, presentation to immune cells, and repair. But when these immune cells are activated too much, they can cause damage to the tissues and promote continued inflammation (17).

Many chronic disorders have comparable inflammatory pathways and mechanisms. TNF, IL-1, and IL-6 are cytokines that are inflammatory that trigger joint inflammation in RA, vascular inflammation in atherosclerosis, and JAK/STAT signaling. Uncontrolled activation of both the adaptive and innate immune systems, inflammasome activation, and neutrophil damage to epithelial cells all contribute to the development of chronic inflammatory bowel disease (CIBD). Microglia stimulation and blood-brain barrier disruptions have been linked to peripheral systemic

inflammation and chronic neuroinflammation, which are increasingly thought to have a role in neurodegenerative diseases such as Alzheimer's (18).

During injury, the inflammatory pathways are essential for a protective function. If left uncontrolled however it will lead to chronic inflammation and be involved in the processes of disease pathogenesis. Shared mediators of inflammation such as NLRP3 inflammasomes, IL-1, macrophages, and neutrophils might be therapeutic targets to reduce the global disease load.

2.2. The molecular mechanisms underlying the inflammatory response

The inflammatory response, a crucial protective defense mechanism executed by the immune system against pathogens, tissue damage, toxins, or other stimuli that pose a threat, is a tightly orchestrated cascade of innate and adaptive immune events mediated by various molecular players including cytokines, chemokines, inflammatory mediators and immune effector cells. While physiological inflammation resolves naturally when the stimulus is removed, failure in regulating inflammatory signaling pathways leads to sustained inflammatory responses, tissue damage and development of chronic diseases. Pattern recognition receptors (PRRs) recognize pathogens and damage, triggering signaling cascades that distribute inflammatory mediators, induce additional immune cells toward the inflamed tissue, and increase stimulus elimination.

2.2.1. Inflammasomes

Inflammasomes, multiprotein complexes located in the cytoplasm of innate immunity cells, are another essential the innate immune system component capable of initiating a powerful inflammatory response. Upon detection of appropriate danger signals they assemble to activate inflammatory caspases, mainly caspase-1. It is therefore involved in integrating sensing of innate immune system with development of inflammatory responses (16).

2.2.2. Pro-inflammatory Cytokines

Cytokines include protein messengers who regulate cell-to-cell communication and immune system response to inflammation. IL-1, IL-6, and TNF - α are major pro-inflammatory cytokines that are rapidly produced by numerous innate immune cells upon identifying patterns of signalling activity, which include the TLR-NF-B pathway and the MAPK pathway (19). Activated macrophages and monocytes produce TNF- α and IL-1, which promote endothelial activation and activation of leukocytes through enhancing the production of adhesion molecules like ICAM-1 and VCAM-1 upon endothelial cell types (20). This causes an inflow of immune cells to wounded or diseased tissue. Furthermore, TNF - α and IL-1 promote inflammation by releasing additional

cytokines, chemokines, nitric oxide (NO), and matrix-degrading enzymes (21). IL-6 has a unique function in maintaining inflammatory responses and boosting the acute-phase reaction seen after infection or tissue damage, as well as in many chronic inflammatory disorders. Chronic production of IL-6 is closely associated with various diseases such as autoimmune disorders, cytokine storm syndromes, cancer progression and cytokine mediated inflammatory disorders (22). Studies in microglial cells showed that LPS induced TNF- α , IL-1, IL-6 through MAPK and PKC pathways and nitric oxide generation has been shown to be essential for IL-1 and IL-6 generation (20).

2.2.3. Chemokines

Chemokines constitute a kind of cytokine whose primary role is to guide the movement and traffic of immune cells like neutrophils and monocytes to inflammation regions in response to inflammatory stimuli (23). This is accomplished by their capacity to attach to certain receptors found on immune cells. Therefore, binding to these GPCRs directs immune cell migration through chemotactic gradients thereby recruiting them to areas of infection or tissue damage. In addition to attracting immune cells, chemokine signaling guides leukocyte adherence to endothelial cells, as well as leukocyte activation and retention in inflamed tissues. Chemokine signaling plays an important role in diseases such as atherosclerosis, various autoimmune disorders, and age-related inflammation, 'inflammaging', where organized chemokine and receptor networks and glycosaminoglycans orchestrate cell migration and immune responses (24). Recent studies have reported additional regulatory mechanisms that finetune inflammation through modulation of chemokine activity and binding such as heterodimeric interactions with galectins which can mediate reduced chemokine receptor activity and hence reduced leukocyte migration, for example, binding of CXCL12 with galectin-3 (25). In addition to cytokines and chemokines, other inflammatory mediators including vasoactive amines like histamine, eicosanoids like prostaglandins and leukotrienes, acute-phase proteins, complement proteins, ROS, and reactive nitrogen species (RNS) play a vital part in the vascular and cellular events that constitute inflammation (26).

2.2.4. Role of Macrophages and Monocytes

Monocytes and macrophages are central effector cells of inflammation and play a vital role in all phases of this response: initiation, amplification and resolution. They are resident macrophages that operate as sentinels and identify inflammatory stimuli through their PRRs, triggering the synthesis of numerous inflammatory mediators, recruiting other immune cells including

neutrophils to the affected region, and orchestrating processes leading to inflammation (27). Macrophages can also adopt different phenotypes and differentiate into a M1 classical or M2 alternative inflammatory macrophage depending on the signals provided by their microenvironment. M1 macrophages are frequently pro-inflammatory because they create reactive species such as nitrogen and oxygen and release cytokines that are pro-inflammatory, resulting in tissue damage and inflammation. M2 macrophages, on the contrary side, have anti-inflammatory capabilities and are in control of tissue repair and settlement by secreting cytokines including IL-10 as well as TGF (28). Persistent stimulation of M1 macrophages has been associated with many chronic inflammatory conditions including atherosclerosis and autoimmune disorders, where continuous cytokine production and ROS cause chronic inflammation and growing tissue damage (21).

2.2.5. Role of Neutrophils

Neutrophils are the earliest and most abundant leukocytes at the site of acute inflammation, having a role in pathogen clearance, inflammation, and wound healing. After recruitment by chemokine gradients, they travel swiftly to the site of infection and injury, where they exert their strong antimicrobial effects via phagocytosis, degranulation, ROS and RNS generation, and the creation of neutrophil extracellular traps (NETs) (29). They develop and distribute pro-inflammatory compounds such as prostaglandins, leukotrienes, and cytokines, as well as matrix-degrading enzymes, which can cause direct tissue injury (17). Hence although essential for eliminating the infectious agent they also contribute to the exacerbation of inflammation and tissue injury, making them key players in inflammatory disease progression. They are heavily linked in inflammatory diseases like atherosclerosis, autoimmune diseases, and inflammatory kidney disease (21).

2.2.6. Role of T Cells

T cells are the primary players in adaptive immune responses, and they perform a crucial part in chronic inflammatory diseases. Antigen-presenting cells (APCs) deliver antigens to native T lymphocytes in the form of co-stimulatory chemicals and cytokines, activating them (27). The cytokine environment controls when activated T cells divide into several categories: Th2, Th1, Th17, Treg, and toxic T cells (30). The inflammatory mediator's cytokines and chemokines are essential for T cell recruitment and polarization through JAK-STAT, MAPK, PI3K, and focal adhesion kinase (FAK) signaling pathways (31). Th1 responses stimulate production of IFN- and drive cell-mediated immunity while Th17 responses drive neutrophilic inflammation and the

development of autoimmunity. Regulatory T cells serve to prevent immune system overstimulation and promote tolerance.

IL-1 family cytokines and IL-18 induced by inflammasome activation are potent inducers of Th1 responses and IFN- production while IL-10 serves to dampen pro-inflammatory responses and promote resolution (16). Abnormal T cell activation as well as an imbalance of pro-inflammatory and regulating subsets are critical in the formation of chronic inflammatory along with autoimmune conditions (29). Finally, inflammatory mediator signaling occurs in intricate systems of innate and adaptive immune signaling channels. PRR engagement activates the NF- κ B pathway, MAPK, and inflammasome pathways, causing the development of cytokines like TNF- α , IL-1, IL-6, and chemokines, who stimulate the activation and recruitment of neutrophils, macrophages, and T cells, perpetuating cytokine and chemokine production, APC stimulation, and Th activation. The regulation of cytokine signaling, chemokine networks, and immune cell polarization is critical for inflammation resolution and chronic disease prevention.

2.3 Key inflammatory signaling pathways

The cellular signaling pathways are a network highly regulated by immune and non-immune stimulations such as pathogens, tissue damage, metabolic signals and immune stimulants to generate inflammation. In the case of acute inflammation, they remove toxic stimulators; however, persistent activation of inflammatory signaling pathways results in long-term inflammation, that contributes towards the pathophysiology of many illnesses, including RA, cardiovascular disease, IBD, asthma, neurological disorders, diabetes, and cancer (32). Still, inflammatory signaling pathways cannot function in isolation; they are linked, redundant, and the inflammatory stimulation continues even when one pathway is blocked.

2.3.1. NF- κ B Signaling pathway

The NF- κ B signaling pathway regulates inflammation and immune responses (30). NF- κ B transcription factors govern immune cell activation, proliferation, survival, and cytokine production in response to inflammation (33). At rest, IBs in the cytoplasm block NF- κ B protein, inhibiting activation. Inflammatory events such as TNF-, IL-1, TLR ligand binding, oxidative stress, and microbial metabolites can phosphorylate and degrade IBs, prompting NF- κ B to migrate to the nucleus along with activation (34). The two most established signaling pathways are canonical and non-canonical NF- κ B activation pathway, respectively initiated by various inflammatory stimulators or the receptors of TNF family members. Activation of the NF- κ B signaling pathway

leads to the production of various inflammatory molecules, such as TNF-, IL-6, and IL-1, as well as chemokines, migration and adhesion molecules. It also increases the transcription of COX-2, iNOS, and antiapoptotic proteins (35). Sustained stimulation through the NF-κB signaling pathway can lead to chronic inflammation, autoimmune disorders, cancer, and neurological problems (36). NF-κB signaling regulates macrophage and monocyte polarization, inflammasome activation, inflammatory cytokine production, and cell survival. It also interacts with other signaling molecules like STAT1 and STAT6 to facilitate context-specific immune responses. Given that NF-κB signaling activates multiple inflammatory molecules simultaneously, this signaling pathway has become an important therapeutic target in chronic inflammation (30).

2.3.2. MAPK signaling pathway

Another important inflammatory signaling mechanism involves the mitogen-activated protein kinase (MAPK) pathway, which controls cellular reactions in response to stress, mediators of inflammation, growth factors, and microbial substances. TLRs, cytokine receptors, physical harm, and oxidative stress together stimulate the three primary MAPK signaling cascades: ERK, JNK, and p38. Activated MAPK kinases phosphorylate and regulate target transcription factors, subsequently mediating the synthesis and secretion of different mediators of inflammation, including pro-inflammatory cytokines TNF-α, IL-1, and inflammatory response such as immune cell proliferation, apoptosis, and tissue remodeling (37). The MAPK pathway can interact with and augment the NF-κB signaling and thereby activate the matrix metalloproteinases (MMPs) and other catabolic enzymes that mediate the destruction of joint tissues and cause the degradation of cartilage and destruction of joint in osteoarthritis, which are considered the chronic inflammatory diseases. The specific role and activation of the three MAPKs in inflammatory signaling are known, for example, p38 MAPK can be stimulated by inflammatory cytokine IL-1 to enhance the synthesis of inflammatory mediator such as TNF-, while JNK signaling pathways can trigger apoptosis and stress-induced inflammation. ERK signaling typically promotes cell growth and survival, which makes it a promising target in a variety of chronic inflammatory diseases and tumor-associated microenvironment (20). The high redundancy among MAPK signaling pathways as well as the essential role in normal cellular physiology pose great challenges to its targeting for effective therapeutic efficacy without adverse effects (38).

2.3.3. JAK-STAT signalling pathway

The Janus kinase-signal transducer and activator of transcription (JAK-STAT) signalling system is another important cytokine-mediated route involved in immunity, inflammation, cell proliferation, and survival (39). The phosphorylation and activation of the STAT protein occurs when a cytokine binds to its relevant receptor and the related JAK kinases are activated (39). Activated STAT proteins eventually dimerize, go to the nucleus, and directly stimulate gene expression. Multiple cytokine receptors are involved in the regulation of chronic inflammatory responses, and the majority of them activate the JAK-STAT pathway. The activated JAK-STAT signaling pathway has been strongly implicated in the development of various inflammatory diseases, including autoimmune diseases (40). STAT3 has been identified as an essential STAT protein in the pathophysiology of inflammatory diseases because it promotes cell survival, proliferation, angiogenesis, and chronic inflammation. What is important is the considerable interaction between the JAK-STAT and NF- κ B pathways. STAT3 can trigger long-term NF- κ B activation, resulting in self-amplification of inflammation in chronic inflammation and the tumor microenvironment. The JAK-STAT pathway is a promising target for treating inflammation and chronic diseases like RA because of its capacity to respond to many cytokine signals at the same time (41).

2.3.4. COX and LOX pathways: Eicosanoid Signaling

Cyclooxygenase (COX) as well as lipoxygenase (LOX) are two major enzymes involved in the production of arachidonic acid metabolites known as eicosanoids (35). These physiologically active lipid mediators have critical functions in controlling inflammatory responses, such as increasing vascular permeability, causing fever and discomfort, and attracting immune cells. COX pathway produces prostaglandins and thromboxanes while LOX pathway produces leukotrienes and lipoxins, which have both potent inflammatory or immunosuppressive activities. Prostaglandin E, a prominent mediator in the inflammatory response generated primarily by COX-2, causes vasodilation, edema, fever and pain hypersensitivity. Leukotrienes stimulate the recruitment of leukocytes into inflammatory sites and play crucial role in allergic inflammatory responses and bronchoconstriction (42).

2.4. Current therapies and limitations of anti-inflammatory medications.

Inflammation is a fundamental defense mechanism that protects the organism against the bacterial infections, tissue injury, and other unwelcome stimuli (2). However, dysregulated or chronic inflammation contributes to the etiology of a various disorders, including RA, IBD, cardiovascular

disease, neurodegenerative diseases, asthma, and cancer. Significant improvements have been made in the treatment of inflammatory conditions over the last few decades, starting with traditional NSAIDs and progressing to increasingly specific and targeted biologic therapies and synthetic drugs. Anti-inflammatory therapies are currently used, but have several limitations, including adverse effects, lack of disease control, resistance, and the complexity of inflammatory signaling pathways. The currently available drugs are mostly directed towards only one mediator or pathway in a highly interconnected inflammatory network, with often limited long-term efficacy and a high risk of side effects.

2.4.1. NSAIDs and Cyclooxygenase (COX) Inhibition

NSAIDs are used worldwide and are frequently prescribed anti-inflammatory drugs because of their anti-inflammatory, analgesic and anti-pyretic effects. They block cyclooxygenase (COX) enzymes, which convert arachidonic acid into prostaglandins and thromboxanes. Prostaglandins are most known as mediators of pain and fever, vasodilation and inflammation; thromboxanes, as important players in the blood coagulation and vascular homeostasis (9).

COX-2, on the other hand, is an inducing enzyme that is highly elevated in inflammation and contributes significantly to prostaglandin production, which is also implicated under inflammation (43). NSAIDs are mostly recognized because of their anti-inflammatory and neuropathic benefits, which are connected to their inhibitory action on COX-2. Many of their adverse effects originate from their capacity to inhibit COX-1 (44).

Common non-selective NSAIDs include ibuprofen, aspirin, naproxen, as well as diclofenac, which inhibit both COX-1 as well as COX-2 enzymes (3). These drugs are effective in reducing pain and inflammation but have a strong association with gastrointestinal toxicity such as gastric irritation, peptic ulceration, gastrointestinal bleeding and perforation when used over a prolonged period. The inhibition of COX-1-derived protective prostaglandins results in impairment of the gastric mucosal barrier and increased sensitivity of the gastrointestinal tract to acid-induced damage (45). In addition, NSAIDs may also affect renal perfusion and electrolyte balance thus causing renal toxicity, hypertension, edema and acute kidney injury, especially for elderly patients or those with preexisting renal disease (46).

Celecoxib and rofecoxib decrease COX-2's inflammatory activity while having little effect on COX-1's anti-gastric effects. While coxibs were proved to have less gastrointestinal side effects than regular NSAIDs, many studies have since showed a greater risk of cardiovascular events such

as heart attack, stroke, myocardial infarction, or thrombotic events (47). The withdrawal of rofecoxib from the market highlighted the significant cardiovascular hazards of selective COX-2 medicines. These harmful effects on the heart and blood vessels are thought to be caused by the unbalanced regulation of prostacyclin and thromboxane, which stimulate blood platelets to clump together and cause blood vessels to constrict (48).

Another drawback of NSAIDs is that they only relieve symptoms, rather than changing the underlying disease process. They work well to help relieve pain and inflammation, but don't stop the damage to tissues or the progression of the disease, as they can do in chronic inflammatory diseases like rheumatoid arthritis. For this reason, it is best to use these drugs at the lowest dose and duration possible to manage inflammation while reducing toxicity (49).

2.4.2. Corticosteroids

Corticosteroids, another significant family of anti-inflammatory medicines, are frequently utilized for treating acute and chronic inflammatory disorders. They have strong anti-inflammatory and immunosuppressive properties, which include lowering gene expression and down-regulating mediators of inflammation like as chemokines, cytokines, prostaglandins, and adhesion molecules (50). Corticosteroids reduce phospholipase A2 activity, which decreases arachidonic acid production along with prostaglandin and leukotriene production. They also inhibit the activation of nuclear factor-kappa B (NF- κ B) signaling, a key pathway for inflammatory gene expression (51).

Because of their wide spectrum of activity, corticosteroids are very effective in quickly controlling inflammation in diseases like asthma, autoimmune diseases, allergic reactions, inflammatory bowel disease, and rheumatoid arthritis. They are also known to have wide systemic effects, though, which can also lead to toxicity, especially with long-term therapy. Long-term corticosteroid usage can cause major adverse effects, including gaining weight, osteoporosis, high blood pressure, diabetes, adrenal gland suppression, cataracts, muscle atrophy, and more infections (35). Patients may also be at risk for opportunistic infections and delayed wound healing due to long-term immunosuppression.

In addition, corticosteroids are not specific to any inflammatory pathway, and thus normal immune functions can also be impaired. Their widespread immunosuppressive activity is a primary reason for their lack of long-term usage, prompting the development of more selective along with targeted anti-inflammatory medicines with improved safety profiles.

2.4.3. Biologics and Targeted Therapies.

In contrast to NSAIDs, which largely treat symptoms, biologics target immune system mediators in the illness process. Biologic drugs such as infliximab, adalimumab, and etanercept block TNF- α , as well as interleukin, B cell, and T cell co-stimulation pathways. TNF- α inhibitors are highly efficient in treating RA, psoriasis, ankylosing spondylitis (AS), and IBD. They reduce inflammation, decrease tissue destruction, and improve patients' quality of life (47). These therapies have proven to be effective in the selective blockade of inflammatory cytokines in the hope that they could induce a marked change in disease outcome and prevent irreversible joint damage.

However, there are still a number of key drawbacks to biologics. They are very specific to some of the cytokines, so that patients may have partial responses because the cytokines are activated in other inflammatory pathways. A large portion of patients don't respond at the first time, or, over time, they become unresponsive. Further, biologics are administered through injection, and can be costly to treat, which can cause difficulties in access to these medicines in many health care systems.

Biologics have immunosuppressive characteristics, making the patient more susceptible to infections caused by bacteria, viruses, and fungi (52). Long-term biologic therapy has been associated with serious complications like tuberculosis, opportunistic infections and risk of malignancy (35). They are also complicated by infusion reactions, autoimmunity and immune related adverse events. However, biologics have been a significant step forward in treatment, but still do not offer a total, or always reliable cure for chronic inflammatory conditions.

2.4.4. JAK Inhibitors and the Problem of Single-Target Therapy

Another key advance in anti-inflammatory drug therapy is aimed at targeted synthetic drugs that inhibit Janus kinase (JAK) inhibitors. JAK inhibitors block cytokine-mediated intracellular signalling pathways that are related to immune activation and inflammation. These agents block the activation and phosphorylation of STAT proteins through blocking the JAK pathway thus blocking several cytokines that play a role in autoimmune diseases.

JAK inhibitors are oral agents that are more effective at blocking multiple cytokines than biologics; however, there are concerns about infections, thromboembolic events, cardiovascular risks, and risks for malignancy. More significantly, the growth and development of their drug have raised a

fundamental issue in anti-inflammatory drug research: the redundancy and complexity of inflammatory networks.

Cytokines, prostaglandins, leukotrienes, chemokines, transcription factors and immune cells are all involved in highly interconnected signaling pathways to regulate inflammation. As a single drug, inhibiting a single mediator (COX-2, for example, or TNF- α) does not always prevent inflammation from occurring, as the body develops other pathways to keep the disease going (53). This redundancy is what makes patients non-responsive to treatment and impedes complete eradication of the disease, while also causing therapeutic resistance and variation in patient responses. As a result, in many cases, patients still suffer from ongoing inflammation despite specific therapy.

2.4.5. Emerging Multi-Target Strategies

The disadvantages of single-target therapies have sparked the interest to develop multi-target anti-inflammatory treatments. Researchers are now investigating multiple drugs that can be used to simultaneously modulate several pathways, without causing excessive toxicity. Double COX-2/5-lipoxygenase (5-LOX) inhibitors, which prevent the formation of prostaglandins and leukotrienes, are one example. Leukotrienes are also frequently seen playing an important part in inflammation and tissue damage, thus dual inhibition would be predicted to be even more effective but less likely to produce gastrointestinal side effects than traditional NSAIDs (54).

In the same way, nitric oxide-releasing NSAIDs (NO-NSAIDs) have also been studied to be safer alternatives to traditional NSAIDs. Such compounds are not only COX inhibitors but also release nitric oxide, which could maintain integrity of gastric mucous layer and enhance the vascular function (55). Other studies involve personalized medicine strategies, drug delivery systems using nanotechnology and natural bioactive compounds with multiple effects on various inflammatory mediators.

In conclusion, present anti-inflammatory drugs are still indispensable in medicine, and have contributed to the management of inflammatory diseases. There are however important restrictions in all therapeutic classes such as gastrointestinal, renal, cardiovascular and immunosuppressive toxicity, and under-dosing and therapeutic resistance. The complicated and redundant inflammatory signaling pathways make the development of completely effective treatments difficult. Thus, safer, multi-target therapies with fewer adverse reactions and anti-inflammatory benefits are increasingly being pursued as potential individual treatment methods.

2.5. The medicinal plants and phytochemicals

Medicinal herbs have traditionally been recognized as key sources of bioactive compounds for the management and treatment of inflammatory disorders (56). As more research on medical benefits of plants have been performed, as phytochemicals are known to have significant anti-inflammatory activities and are typically less likely to induce side effects than nonsteroidal anti-inflammatory medicines (NSAIDs) or corticosteroids (57). The molecular targets and inflammatory mediators that they act upon are less likely to be one and the same, and are often multiple, as opposed to conventional synthetic drugs that target one specific molecule. These pathways include NF- κ B, MAPK, PI3K/AKT, JAK/STAT, and Nrf2, in addition to inflammatory enzymes like COX and LOX, and pro-inflammatory cytokines (58). In reality, medicinal plants have several pharmacological effects, emphasizing their usefulness in chronic inflammatory and multifactorial disorders.

2.5.1. Importance of herbal medicine

Medicinal herbs have been utilized for centuries to prevent and heal illnesses in traditional therapeutic systems like Ayurveda, TCM, and Kampo medicine. The theory of herbal medicine is based on the medicinal qualities of plants that contain a range of active chemicals, including anti-inflammatory, antioxidant, immunomodulatory, as well as antibacterial effects (59). According to worldwide health surveys, plant-based remedies continue to play a key role in many people's health care systems, particularly in developing countries, as their primary prescription. Apart from this, many modern drugs are directly extracted from plants or are based on plants derived lead(60).

Herbal medicine is one such medicine which has a unique mode of action and contains a combination of multiple phytochemicals that produce the therapeutic effect instead of any single active molecule (61). The synergistic effect is particularly beneficial in cases of chronic diseases with multiple molecular mechanisms and complex inflammatory networks.

2.5.2. Secondary metabolites anti-inflammatory properties.

Secondary metabolites of plants are compounds that have biological activities, some of which are mainly defence mechanisms and adaptation, and others have strong pharmacological activities in humans. Some of these metabolites possess considerable anti-inflammatory and antioxidant effects (62). These substances regulate pro-inflammatory cytokines as that include TNF- α , IL-1 β , and IL-6, along with important signaling pathways like NF- κ B, MAPK, PI3K/AKT, Nrf2, JAK/STAT, and the NLRP3 inflammasome (63). In addition, phytochemicals have the ability to inhibit

inflammatory enzymes such as COX-2, 5-LOX and inducible nitric oxide synthase (iNOS) and decrease oxidative stress, which could result in anti-inflammatory and cytoprotective effects.

2.5.3. Major Classes:

2.5.3.1. Polyphenols

Anti-inflammatory and antioxidant activities are frequently stated as their key pharmacological effects (58). This category includes flavonoids, stilbenes, and phenolic acids, several of which have been shown to be potent regulators of inflammatory signaling pathways. Polyphenols also influence the activation of inflammasomes and oxidative stress pathways, that are responsible for the observed protective benefits in metabolic conditions, cardiovascular illnesses, and neurodegenerative disorders (64).

2.5.3.2. Alkaloids

They are nitrogenous secondary metabolites present in many medicinal plants, have been identified as promising candidates for anti-inflammatory drugs (65). Several alkaloids, such as colchicine, berberine, and mitraphylline, have anti-inflammatory properties, including the ability to inhibit nitric oxide production, inhibit pro-inflammatory cytokine production, modulate inflammasome activation, and regulate microtubule dynamics (65). Alkaloids can inhibit COX and LOX enzymes, as well as NF- κ B signaling pathways, making them effective anti-inflammatory and immunomodulatory drugs (61). Because of their various modes of action and complexity, alkaloids are thought to be promising prospects for anti-inflammatory medication development.

2.5.3.3. Terpenoids

Terpenoids are one of the most significant and chemically varied groups of natural compounds, with several having strong anti-inflammatory activities (66). The monoterpenes, sesquiterpenes, diterpenes and triterpenes like borneol, escin, parthenolide and curcumol are included in this class (67). Terpenoids have anti-inflammatory properties via inhibiting NF- κ B and MAPK signaling pathways, reducing COX-2 as well as iNOS production, suppressing Fc ϵ RI-dependent mast cell degranulation, and modulating cytokine levels (68). The class of terpenoids is full of possibilities for anti-inflammatory targets and experimental models because of the 281 terpenoids found in the systematic review.

2.5.4. Multi-target Nature of Phytochemicals.

One of the defining features of phytochemicals is that they have multiple targets/polypharmacological activity. Phytochemicals are likely to affect more than one molecular

pathway while single-target synthetic drugs are more likely to act on one pathway. Several flavonoids and phenolic acids, terpenoids and other phytochemicals from dietary plants have been shown to connect together in network pharmacology studies to form major inflammatory pathways including NF- κ B, MAPK, PI3K/AKT, Nrf2, COX-2, TNF- α , and AKT1 (69).

Plant extracts and phytochemicals affect immune cell signaling pathways such as NF- κ B, MAPKs, AP-1, STAT1/6, IRFs, PPAR γ , KLF4, and PI3K/AKT, polarizing macrophages towards pro-inflammatory (M1) or anti-inflammatory (M2) phenotypes (63). Natural products target inflammatory cytokines, as well as important signalling pathways. These compounds also have anti-oxidative and rebalance the microbial balance in dysbiosis-associated inflammatory diseases (70).

Another critical therapeutic approach is the dual COX/LOX inhibition exhibited by phytochemicals like tannins, flavonoids, alkaloids, terpenoids and plant steroids. Combining these routes may be more successful in lowering inflammation produced by eicosanoids and have fewer negative effects than using selective COX inhibitors alone (62). The multidimensional and polypharmacological nature of medicinal plants is their main reason for being a great potential source for the treatment of complex chronic inflammatory diseases. It also poses challenges of standardization, bioavailability, pharmacokinetics and mechanistic interpretations in the development of phytochemical-based therapeutics (58).

2.6. Columbin: Sources, Chemistry and Pharmacological Profile

Columbin has a diterpenoid molecule who has recently been shown to have a number of pharmacological activities, including anti-inflammatory characteristics, and is gaining popularity as a naturally occurring chemical (71). It is classified as a furanolactone or cis-clerodane diterpene and is mainly associated with medicinal plants belonging to the genera *Tinospora* and *Jateorhiza*. Columbin has been identified as a significant bioactive element accountable for several of the medicinal benefits of these plants. It has long been used in conventional healthcare systems to manage inflammation, fever, infections, and other diseases (10,72–74). However, in recent years, the mechanisms of columbin's bioactivity, such as interactions with the cyclooxygenase (COX) enzyme and nitric oxide (NO) related inflammatory pathways, have been increasingly investigated.

2.6.1. Botanical classification of plants and chemical classification.

Columbin is obtained mostly from root tissue of *Jateorhiza columba* and a range of *Tinospora* species, including *Tinospora capillipes* and *Tinospora sagittate* (72). Moreover, for the first time

purified columbin was reported from *Tinospora bakis*, which further broadened the range of the phytochemistry of this diterpenoid in the *Tinospora* genus (12). The diterpenoid furanolactones and clerodane derivatives like columbin have been identified consistently in reviews on *Tinospora* species as the phytochemicals responsible for various medicinal effects like anti-inflammatory and antimicrobial activities (73,74). The occurrence of columbin derivatives in medicinal plants emphasizes the relevance of this family of chemicals in ethnopharmacology and natural medicine development.

2.6.2. Structure and class

Chemically, columbin is categorized as a furanolactone or cis-clerodane diterpene with a diterpenoid skeleton, including a furan ring and a lactone group, which are typical motifs found in bioactive compounds (72). It is a member of the larger class of diterpenoid lactones and neoclerodane diterpenoids found in other medicinal plants, such as *Tinospora* species (73). Each member of this family has a diverse set of pharmacological properties, comprising anti-inflammatory, antibacterial, antioxidant, cytotoxic, and immunomodulatory activities. The structural properties of columbin also make it a significant lead compound in phytochemical and pharmacological studies because of its capacity to interact with inflammatory enzymes and signaling pathways.

2.6.3. Pharmacological activities

Columbin and columbin rich plant extracts have been reported to possess several pharmacological activities as derived from traditional medicine uses as well as experimental studies. It is one of the most commonly documented activities due to its anti-inflammatory properties and analgesic qualities. Traditionally, medicinal roots with columbin have been used as analgesics, antipyretic, anti-inflammatory, anti-bacterial, antiulcer, anti-tumour and anti-stress agents (72). Further experimental investigations have confirmed anti-inflammatory and cytotoxic activity of columbin alone, which is consistent with traditional use of *Tinospora* and *Jateorhiza* species, as mentioned above (74).

Neoclerodane diterpenoids and furanolactones derived from *Tinospora* and *Jateorhiza* species have exhibited antimicrobial activity. The properties of these compounds indicate that they have inhibitory activity against different microorganisms, which suggests their possible use in plant protection and therapeutic antimicrobial applications (72). Furthermore, in various pharmacological studies, *Tinospora* species with diterpenoid lactones such as columbin have

exhibited antioxidant, hepatoprotective, immunomodulatory, and cardioprotective properties when tested as extracts(75,76). In the available literature however, the biological activities have been shown for whole plant extracts and diterpenoid rich fractions of the plant, but direct evidence of the antioxidant or hepatoprotective effect of isolated columbin has been limited in the evaluated studies.

2.6.4. Evidence on COX Inhibition and Inflammatory Modulation

Much study has been conducted on columbin's anti-inflammatory potential, specifically its impact on the enzyme cyclooxygenase and nitric oxide generation. *Tinospora bakis* pure form of columbin inhibits COX-1 and COX-2 enzymes in vitro around doses comprising 63.7% and 18.8%, correspondingly, at 100 μ M. (10). Molecular docking studies also indicated that the COX-2 enzyme showed more affinity toward columbin, due to the binding interactions between certain amino acid residues and columbin, such as Tyr385 and Arg120, which are essential for a binding interaction with arachidonic acid and for hydrogen abstraction during prostaglandin synthesis.

Columbin reduces nitric oxide (NO) synthesis in LPS/IFN- γ activated RAW264.7 macrophage cells while avoiding cytotoxicity or cell death. Columbin's anti-inflammatory action may be mediated by COX and nitric oxide pathways, doesn't prevent NF- κ B nuclear translocation (10). Furthermore, in-vivo studies show anti-inflammatory potential of columbin in animal models, showing its effectiveness in decreasing carrageenan-induced paw edema, which is a significant activity under physiological conditions. Remarkably, there was no apparent toxicity in mice and human liver cell models, which confirmed the initial safety profile of columbin as an anti-inflammatory lead compound (10).

In addition to studies with isolated columbin, research carried out with extracts of *Tinospora* and its associated diterpenoids indicate that this phytochemical group have wider inflammatory pathway modulation properties. These compounds and extracts have been shown to reduce inflammatory mediators. They also affect signaling pathways including JAK/STAT pathway and the MAPK pathway (77). These results suggest that columbin-associated diterpenoids might possess immunomodulatory and anti-inflammatory effects in addition to COX inhibition.

Hence, columbin is a biologically significant furanolactone/cis-clerodane diterpene mainly found in *Tinospora* and *Jateorhiza* spp. At the moment, data suggests that it has anti-inflammatory properties, which are connected to its ability to control nitric oxide synthesis, cyclooxygenase activity, and diminish acute inflammatory responses within in vivo studies all with little apparent

toxicity. Collectively, extract level studies on *Tinospora* species revealed additional antioxidant, antimicrobial, hepatoprotective, immunomodulatory, and cardioprotective properties, but there is limited direct evidence of these properties at the compound level and they warrant further detailed pharmacological investigation.

2.7. Network Pharmacology in Drug Discovery

The idea of network pharmacology has appeared as a major strategy in modern drug discovery from the perspective of systems biology, stepping away from the “one drug – one target” paradigm. This method can be especially useful for the investigation of diseases of multiple causes, such as herbal medicine, in which multiple biological pathways are involved at the same time (78–80). Network pharmacology differs from traditional pharmacology, which typically targets a specific molecule, by looking at disease as a disruption of a network of molecules and examining the effect that pharmaceutical compounds have on multiple molecules and pathways simultaneously. Therefore, it plays a more and more significant role in systems biology, precision medicine, phytopharmacology and natural product based therapeutic research.

2.7.1. Concept and Principles of Network Pharmacology

Based on the definition, network pharmacology is a multidisciplinary research topic that combines system biology, bioinformatics, computational biology, and pharmacology to create and evaluate drug-target-disease interaction networks. Its primary objective is to acquire understanding into the systems-level processes underlying the illness and to create treatment ways that address the underlying causal networks, as opposed to just suppressing symptoms (79,80). Similarly, complex illnesses such as cancer, cardiovascular disease, neurodegenerative disease, and metabolic syndromes are triggered by disruptions in entire biological networks rather than single molecular abnormalities.

The basic principle of network pharmacology is to search for targets associated with the disease, connect the targets of the drug molecules to the biomolecular interaction network, and analyze the topology of the network to find important “network targets” that might be used for the treatment of the disease (81,82). Researchers usually combine information from genomics, transcriptomics, proteomics, metabolomics, and pharmacological databases to build protein–gene–signaling pathway–metabolome–drug interaction maps. These networks are then analyzed computationally to identify key nodes, hubs, pathways and interaction modules that are associated with disease progression or therapeutic response. Network pharmacology is used to explore the effects of

pharmaceuticals on several connected pathways, which may aid in better comprehending the drugs' mechanisms of action as well as potential synergies.

2.7.2. Multi-target Therapeutic Approaches

Multi-target therapeutic strategies is one of the main achievements of network pharmacology. While the study of single-target drugs has been successful for diseases where one molecular abnormality is largely responsible, it is more often than not unsuccessful for complex multigenic and multifactorial diseases. Network pharmacology thus facilitates multi-target drugs, combination therapies and synergistic cocktails for modulation of multiple disease pathways (83–85).

Within this framework Hopkins suggested a number of strategies such as rational multidrug combinations, multi-component therapeutics, and single molecules specifically engineered to bind to multiple biological targets. Of these, single multi-target molecules have real-world benefits such as reduced drug burden and potentially better compliance (83). As part of the concept of polypharmacology, “designed promiscuity” is the intentional optimization of therapeutic compounds to interact with beneficial targets and to have minimal interaction with antitargets, and to have minimal off-target toxicity (86,87). These strategies are particularly applicable in diseases involving compensatory signaling pathways, drug resistance, and/or dysregulation at the network level.

2.7.3. Application in Herbal Medicine and Natural Products

Herbal medicines and natural product research are ideal applications for network pharmacology, due to the fact that herbal formulations are naturally multi-component, multi-target and multi-pathway. Many bioactive compounds are present in traditional herbal medicines and Traditional Chinese Medicine (TCM) formulations and act synergistically to achieve therapeutic effects by modulating biological networks (88,89). Thus, network pharmacology notions are strikingly comparable to the holistic therapeutic conceptions of traditional medical systems.

The most common network pharmacology research method is to collect and screen phytochemical constituents of medicinal plants or formulations. Pharmacological databases and computational techniques are then used to predict potential targets for these compounds. Then disease associated targets are identified from public databases and transcriptomic studies and then targets common to both compounds and disease are identified. These overlapping targets are then added together to create herb–compound–target networks (H–C–T), drug–ingredient–target–pathway–disease

networks (DITPDD), or compound–target–pathway networks (90,91). Afterward, functional enrichment analysis is conducted to find out the pathways that are biologically relevant, and in many cases the predicted interactions are validated by molecular docking, in vitro and in vivo assays.

Network pharmacology is currently employed in numerous parts of TCM, including as molecular mechanisms, identifying active phytochemical components, herb compatibility, predicting toxicity and safety, and medicine repurposing (91). These approaches offer scientific basis for understanding the therapeutic complexity of the herbal formulations and help in evidence-based phytopharmacological studies.

2.7.4. The identification of Compound–Target–Pathway Interactions

One of the key goals of network pharmacology is to find and analyze compound–target–pathway interactions by combining bioinformatics databases and network analysis tools (88). This usually entails initial prediction of bioactive compound to molecular target, followed by target to biological pathway and target to disease phenotype. There are several types of biological interaction networks, with PPI networks, compound-target networks, compound-target-pathway networks, and compound-target-disease networks. Visualization and evaluation of these complex networks are frequently done using software platforms like Cytoscape (92).

Many studies have employed the “drug–ingredient–target” (DIT) and “component–target–pathway” (CTP) networks to reveal important active ingredients and signalling pathways of TCM formula. The interactions predicted are frequently confirmed by molecular docking studies and models to confirm target engagement and therapeutic activity (90). Moreover, transcriptomic response profiling has been combined with network pharmacology methods to model the modulation of global pathways and provide predictions of combination therapies that can target multiple pathways, thus enhancing the therapeutic efficacy of natural products (93).

In general, network pharmacology can serve as a systems-level perspective of diseases as perturbations of networks and therapeutic agents (especially herbal medicine and natural products) as multi-target modulators of the networks. Network pharmacology can be used to design and validate more rational multitarget drugs, explain the mechanism of complex herbal medicines, and shorten the time and effort required to identify or re-purpose new drugs that can target multiple components of coordinated biological signaling modules.

2.8. Application of molecular docking in pharmacological and phytochemical study.

Molecular docking is a critical computational tool for predicting at molecular level the interaction among a tiny molecule and biological target. It has emerged as a fundamental instrument for contemporary pharmacology and phytochemical research, aiding in the validation of therapeutic targets, virtual screening, lead optimization, and the study of mechanisms. Recently, combining docking methodologies with AI, molecular dynamics (MD) simulations, network pharmacology, and experimental assays has emerged as the preferred way for improving the dependability and efficacy of drug development pipelines. One important application of docking is of phytochemicals because medicinal plants may be rich in multiple bioactive compounds which act on more than one biological target, and computational screening is of great value to identify phytochemicals of therapeutic interest.

2.8.1. Principles of molecular docking

The fundamental purpose of molecular docking is to define the binding process and affinity of a given ligand for the active site of a target protein (94–96). Docking simulations try to replicate spatial and kinetic aspects of the interaction involving protein binding pocket with a ligand while also assessing stability of the resulting protein-ligand complex. The docking technique has two key components: the search algorithm along with the scoring function. Search algorithm is used to sample various ligand orientations, positions and conformations inside the target binding pocket until a conformation with an energetically favorable binding is found (94–96). Various methods for efficiently sampling conformational space are used in different docking software packages, including genetic algorithms, Monte Carlo and stochastic optimization methods.

The second significant element is the scoring function, which is applied to predict the strength of protein-ligand interactions as well as score the resulting poses based on predicted binding affinity. There are different types of scoring functions, all of which try to approximate the binding free energy, based on different principles of computation: physics-based, empirical, knowledge-based, or machine learning algorithms (97,98). Docking simulations are affordable and fast, thus they are frequently employed in virtual screening procedures to study huge chemical libraries during the initial stages of drug development (99).

2.8.2. Protein–Ligand Interaction Analysis and Target Binding Validation.

Molecular docking is a typical method for investigating protein-ligand interactions and determining a target's probable binding processes. Docking poses can help researchers uncover probable binding sites, important residues of amino acids, and chemical reactions, including

hydrogen bonds, hydrophobic contact, electrostatic interaction, π - π stacking, and van der Waals forces, among others. The structural visualization and interaction profiling are typically carried out with the help of the tools PLIP and ProteinsPlus (100,101), which can provide detailed information on protein–ligand complexes.

Docking tests also help determine the binding affinity along with estimate the position of the ligands in the protein's active site; however, orientation prediction is typically more exact than affinity ranking (102). To increase the precision of computational predictions, experimental techniques like surface plasmon resonance (SPR) are commonly utilized to directly examine the binding specificity, affinity, or kinetics of a protein-ligand interaction. A key strength of SPR is that it can be applied to screen even crude plant extracts for biologically active molecules that bind to proteins, making it useful to connect computational and experimental validation approaches (103).

Another key element of docking workflows is protocol validation. A redocking experiment is often conducted on a set of crystal structures, in which the crystallographic ligands are re-docked into the native structure using the same docking method to see if the docking method can successfully re-dock the ligands in the correct orientation. RMSD is used to compare the predicted and actual poses, and is a common measure of the accuracy of docking (104).

2.8.3. The importance and applications in Phytochemical Research.

Molecular docking is an important tool in phytochemical and natural product research since it can provide molecular connections between phytochemicals and protein targets related to disease. Medicinal plants possess a variety of secondary metabolites that may show multiple targets, thus docking is a quick and effective method in order to identify the metabolites that may be used for medicinal role.

An example of the latter is that several phytochemicals were found to bind cancer associated proteins and glycoenzymes when a combination of phytochemical–glycoprotein interaction networks was integrated with AutoDock Vina docking. The best compounds were then matched to the experimental cytotoxic activity of extracts from plants in lung cancer cells, thus linking computational predictions to experimental observations (104). In the same way, docking with the proteins serum albumin and plant antiplasmodial compounds was performed to identify the binding site, conformational change, type of forces involved in the interaction (hydrogen bond,

van der Waal interactions, etc), strength and stability of the interaction. These results provided the optimization of the phytochemical leads for the development of antimalarial drug (105).

Experimental methods like SPR are also used in phytochemical studies to confirm the predictions of a docking. Computational docking and experimental assays can be used together to validate target engagement, and SPR can be applied to screen crude herbal extracts for protein binding molecules, highlighting the potential for computational tools in enhancing the reliability of natural product research results (103).

Taking all of this into account, molecular docking is a rapid and affordable computer technique that can help anticipate protein-ligand interactions and perform virtual screenings of large chemical libraries. Docking studies are essential for understanding the process of binding to a target and discovering new therapeutic medications because they combine search algorithms, scoring functions, interaction analysis tools, and validation techniques like as redocking and SPR (106). Molecular docking is an important tool in the phytochemical research field, acting as a link between plant secondary metabolites and molecular pharmacology, which can be applied in multi-target network pharmacology, finding biologically active phytochemicals, and designing plant extract and natural product studies to test for therapeutic uses.

2.9. Transcriptomic Validation and Bioinformatics Approaches in Target Discovery

The ability of transcriptomic and bioinformatics pipelines to convert high throughput gene expression information into biologically relevant markers and drug targets is key to the discovery of modern therapeutic targets. Transcriptomic data from public archives such as Gene Expression Omnibus (GEO) are widely regarded as main data sources for many disorders, that include cancer, autoimmune disorders, cardiovascular diseases, and neurodegenerative diseases. GEO not only includes the expression profile of miRNAs but also those of RNA-Seq from various tissues, disease stages, and experimental conditions, enabling the identification of disease-specific molecular signatures (107–111). In most studies, the datasets are carefully chosen and are compatible with particular platforms, they encompass samples of a specific tissue type (e.g. tumor vs. normal), and they have a sufficiently large sample size for the statistical power necessary for sound conclusions. To reduce the technical variation and enhance the reproducibility, the sample data is pre-treated by background correction, normalization and batch-effect correction before the downstream analysis (107,108,111).

2.9.1. Gene Expression Datasets and GEO

One of the most important analytical components of these pipelines is Differential Gene Expression (DGE) analysis, which entails looking for genes that are highly up or down regulated in sick and healthy samples. DGE analysis can be used as a first cut approach to discover potential therapeutic targets and disease biomarkers (112–114). For RNA-seq and microarray data, popular bioinformatics tools are DESeq2, edgeR, and limma-voom, which typically use statistical correction methods like False Discovery Rate (FDR) adjustment to minimize false-positive results (107,112–114). More advanced approaches have also been developed, such as those that adjust fold changes for the natural variability in the dosages of genes, and focus on biologically relevant genes rather than genes that look significant merely on the basis of their dosage variability (113). GEO-based DGE analysis has been the initial step in hundreds of disease-focused investigations for identification of disease-associated genes and therapeutic candidates, including those for atrial fibrillation (AF), AD, RA, small-cell lung cancer (SCLC), NSCLC, ischemic stroke, GBM, and other cancers (69,108,111,115–120). In several processes, the detected DEGs are utilized to select clinically relevant genes by integrating machine learning methods, weighted gene co-expression network analysis (WGCNA), or PPI network analysis to get insight into hub genes. For instance, study of AF, AD and RA had combined GEO multi-cohort analyses with machine learning and WGCNA to identify critical disease-associated genes, while the study on NSCLC, GBM and SCLC used DEGs common to multiple GEO datasets and PPI analysis to determine hub genes involved in tumor progression (108,115,119).

Following DGE analysis, functional enrichment analysis is commonly used to obtain understanding into functional importance of discovered genes. Enrichment studies, such as Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG), Reactome, and Disease Ontology (DO), have been widely utilized to characterize DEGs by their biological processes, molecular functions, signaling pathways, and disease correlations, respectively (114,115). Pathway enrichment and pathway impact are commonly used to assess pathways and several computational tools have been widely adopted such as SPIA, Enrichr, and ToppFUND. The analyses revealed certain pathways that were dysregulated in OA, including those related to inflammation, extracellular matrix remodeling, immune response, oxidative stress, metabolism, and cell signalling, which provide insights into the mechanism of disease (120,121). Importantly, enrichment analysis can also be used to determine potentially druggable pathways, allowing the

identification of potentially druggable pathways between transcriptomic analysis and translational therapeutic development.

2.9.2. Validation of Predicted Therapeutic Targets

Another crucial step in the transcriptomic target discovery pipelines is the validation of the targets that are predicted, usually done in silico. The in-silico validation is sometimes performed by cross-dataset validation of candidate genes in other GEO datasets or in cancer data repositories like The Cancer Genome Atlas (TCGA). Also, online tools like TNMplot.com are used to compare expression of genes across normal, tumor, and metastatic tissue (118,122). In addition, researchers include PPI networks, WGCNA, survival analysis, and machine learning models to prioritize hub genes with prognostic and/or therapeutic importance (119,123). Moreover, an increasing number of druggable gene ranking and identification methods such as pathway to target (PITA) and pathway2targets (P2T) are used, as well as drug-to-gene interaction databases such as DrugBank, DGIdb, CMap, DSigDB, and OpenTargets. Also, pathway to target (PITA) and pathway2targets (P2T) are used to rank the druggable genes and identify the therapeutic drugs that may be repurposed, and drug to gene interaction databases such as DrugBank, DGIdb, CMap, DSigDB and OpenTargets are increasingly being used.

Experimental validation verifies computationally predicted targets. A vast number of studies validated the candidate genes at transcript and protein using quantitative real-time PCR (qRT-PCR), Western blotting, and immunohistochemistry in clinical tissues or disease models (120). A functional assay is also performed to assess the role of a specific gene in disease process, proliferation, apoptosis, migration, or in immune regulation. To explore the regulation of genes and therapeutic efficacy of drugs or natural products under physiological conditions, researchers sometimes validate in vivo animal models, such as ischemic stroke and cadmium-induced lung adenocarcinoma (LUAD) models (121). This integrated transcriptomic and bioinformatic workflow together offers a systematic approach that connects gene expression signatures identified in GEO with mechanistic pathway analysis, therapeutic target prioritization, and experimental validation, which ultimately helps to expedite biomarker identification and translational drug development.

2.10. Research Gap and Rationale of the Present Study

Despite tremendous advances in the identification of novel anti-inflammatory drugs, chronic inflammatory disorders continue to be an enormous burden on world health, owing to the disease's

complex biology and the limitations of current treatment strategies. Therapies used to treat inflammation generally target one inflammatory mediator or pathway at a time, such as NSAIDs, corticosteroid and biologics. These therapies are operative against inflammation, but may also have serious side effects, be very expensive, cause suppression of the immune system, or provide only partial relief (124,125). There is now compelling data to suggest that chronic inflammatory diseases are complex and mediated by multiple cytokines, transcription factors, signalling proteins and immune cells that may require a multifaceted therapeutic approach to control the disease long term (126). As a result, the identification of multi-target therapeutic agents that are able to modulate interconnected inflammatory pathways simultaneously, is growing in interest.

The structural diversity and multiple molecular targets of natural products and phytochemicals have made them promising leads in anti-inflammatory drug discovery. Of these, columbin is a diterpenoid furanolactone compound found in medicinal plants of the Menispermaceae family such as *Tinospora cordifolia* and *Jateorhiza palmata* and it has shown anti-inflammatory properties in preliminary experimental studies (127,128). Some previous reports have demonstrated that columbin has inhibitory properties towards cyclooxygenase enzymes and may reduce inflammatory responses by inhibiting pro-inflammatory mediators(12). Besides its anti-inflammatory activity, columbin has also been reported to have an antioxidant, antimicrobial, hepatoprotective, and anticancer effect, which further implies its pharmacological relevance (129). But, although these observations are encouraging, current research on columbin is confined mostly to individual biochemical assays and initial pharmacological studies.

Practically all of the past studies have been on individual proteins or on specific pathways, rather than on network-level interactions that occur in inflammatory diseases. Inflammation is a very complex process, with multiple biological networks working together; therefore, a systems-level approach to study the multi-target mechanisms of phytochemicals is required that would allow the integration of protein interactions, pathway enrichment, and gene expression analysis (130).

With the advancement of computational biology, particularly network pharmacology and molecular docking technologies, effective tools for investigating complicated processes of bioactive substances have emerged. Network pharmacology can help find compound-target-pathway linkages and comprehend multi-target therapeutic processes in biological systems (131). The molecular docking confirms these findings by establishing the binding affinity and interaction of phytochemical as well as target protein. Furthermore, the use of publicly available

transcriptomic data sets to validate predicted targets by correlating with disease-related differential expression profiles provides an additional layer of biological validation (132). Combination of these computational methods has been increasingly valuable in the modern day of drug discovery, especially for plant-derived compounds that possess pleiotropic pharmacological effects.

Thus, the current study attempted to completely investigate columbin's anti-inflammatory impact using network pharmacology, molecular docking, and transcriptome verification. This research aims to identify primary molecular targets, biological processes, along with interaction networks involved with columbin-mediated anti-inflammatory effects. This research will provide a systems-level understanding of columbin's mechanism of action, potentially leading to its development as a multi-target therapeutic agent for chronic inflammatory diseases, as well as a computational approach that can be used to investigate other phytochemicals.

CHAPTER - 3

MATERIALS AND METHODS

Figure 1 depicts the overall study design and analytical workflow for this research. The workflow consists of target identification, network pharmacology analysis, molecular docking, validation, and ADMET prediction.

3.1. Identifying columbin and inflammation-related targets.

PubChem was used to gather chemical information on Columbin (PubChem ID: 188289)(75). SwissTargetPrediction (probability > 0.1), SuperPred (probability $\geq$ 50%), TargetNet (probability $\geq$ 0.5), and PharmMapper (normalized fit score $\geq$ 0.7) were used to predict protein targets using default or recommended thresholds (133–136).

To generate a unique list of targets, all anticipated targets from each resource were combined and duplicates were deleted (137,138). The microarray the data set GSE55235 was also downloaded from the Gene Expression Omnibus. The collection contains synovial tissue samples collected from RA individuals as well as healthy controls. The original data obtained through the Affymetrix Human Genome U133 Plus 2.0 Array was modified and normalized using the Robust Multi-array Average (RMA) approach (139). The differential expression analysis was carried out using limma program, with empirical Bayes moderation. P-values were corrected using the Benjamini-Hochberg method. Genes having $|\log_2 \text{ fold change}| > 1$ and $\text{FDR} < 0.05$ was considered as significantly differentially expressed. Such genes were incorporated to identify prospective columbin targets (140). They were further visualized using a volcano plot and combined with disease-related genes (139).

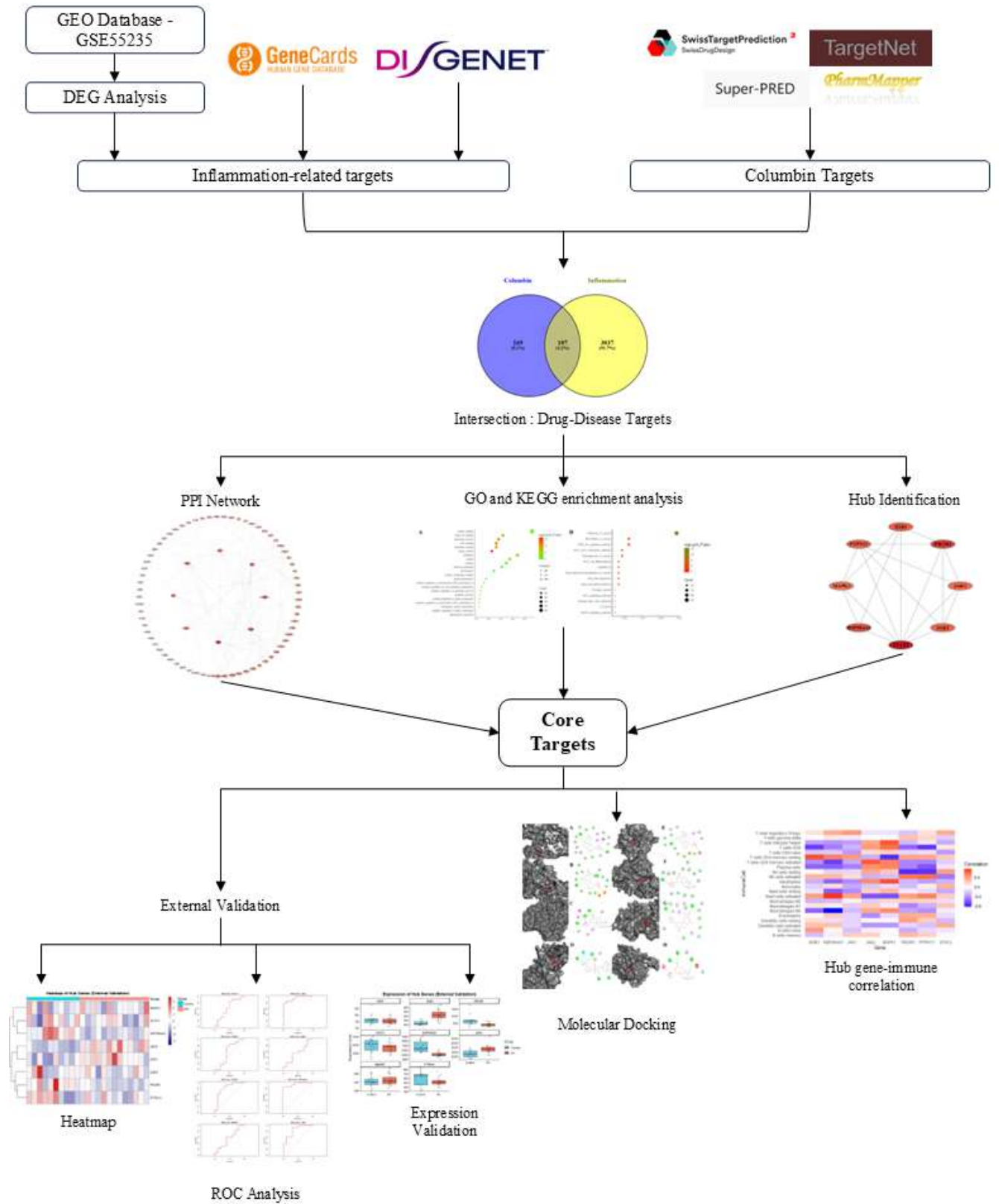


Fig. 1: --- Flow diagram for a network-based pharmacological research.

3.2. Target Overlap Identification and PPI Network Development

Columbin targets (Set A) were predicted by four servers (SwissTargetPrediction, TargetNet, PharmMapper, and SuperPred), and the results were pooled to form a unique set (n=169). Genes related to inflammation (Set B) were sourced from GeneCards and DisGeNET, while genes with differential expression from GSE55235 (Set C) were identified with $|\log_2FC| > 1$ and $FDR < 0.05$. Potential therapeutic targets were discovered as the overlap $A \cap (B \cup C)$ from Venny (v2.1) (141). The PPI networks was made with STRING (v12.0) database. To ensure accurate anticipated interactions and coverage of networks, a high confidence interaction value of ≥ 0.700 was used (142). All evidence sources were evaluated employing STRING's default integrative scoring system. PPI network has been visualised using Cytoscape (version 3.10.4). The CytoHubba plugin predicted hub genes in the static network using five criteria: degree, proximity, maximal clique central (MCC), betweenness, and density of maximum neighborhood components. Top 10 genes from the algorithm were picked, and those found in at least three of the five approaches were recognized as hub genes. In the end, eight hub genes have been identified (143).

3.3. Biological Function and Pathway Enrichment Analysis

Functional enrichment analysis was carried out with the DAVID 2025 database (144). To evaluate genes' functional roles, Gene Ontology (GO) analysis, which comprised Molecular Function (MF), Biological Processes (BP), and Cellular Components (CC), was paired with KEGG pathway enrichment analysis. The Benjamini-Hochberg test was utilized to determine statistical significance, using multiple testing adjustments. Terms having adjusted p-value (FDR) < 0.05 showed substantial enrichment.

3.4. Ligand and protein preparation

To identify the binding location and quality for molecular docking, structures co-crystallized with the ligand (holo) with resolution ≤ 3.0 Å were selected. The protein was prepared using AutoDockTools, which removed water molecules, added polar hydrogens, and assigned Gasteiger charges. Columbin was extracted from PubChem, converted to PDB format, and the energy was reduced using the MMFF94 force field in Open Babel (145). Network study showed PIK3R1 as a hub gene. However, PIK3R1 (p85 α regulatory subunit) forms a heterodimeric complex and PIK3CA (p110 α catalytic subunit) to generate functional class I PI3K. Because PIK3R1 lacks intrinsic catalytic activity and there is no experimental evidence that it is a target of small molecule inhibitors, molecular docking was conducted using the PIK3CA-PIK3R1 heterodimer structure

(PDB: 7PG6) to assess columbin binding to the biologically relevant PI3K complex. This methodology aligns with existing PI3K inhibitor screening approaches, which assess drugs in contrast to the catalytic domain of the functional heterodimer (146).

3.5. Protein–Ligand Binding Analysis Through Molecular Docking

Docking was done using the AutoDock Vina program (v1.2.7). The grid box was positioned on coordinates of the crystallized ligand and designed to entirely surround the binding pocket with a appropriate margin (147). The grid box centred on the co-crystallized ligand and had a binding pocket size of 20×20×20 angstrom. An exhaustiveness value of 32 was chosen to increase accuracy and reliability of ligand conformational sampling at the site of binding. The optimum conformation was chosen determined by its lowest binding energies. The interactions were examined using PyMOL and Discovery Studio (148,149). The docking procedure was verified with redocking the co-crystallized ligand and calculating the RMSD values. The binding energy was verified by cross-validation with SwissDock (150).

3.6. Characterization of Immune Cell Infiltration

The evaluation of relative abundance of immune cell types synovial tissue samples was done using CIBERSORT and LM22 signature matrix, which identifies 22 immune cell groups (151). We used CIBERSORT on the discovery dataset (GSE55235) with 1,000 permutations and only kept samples with $p < 0.05$, suggesting reliable deconvolution. To prevent false positives resulting from excessive multiple tests, we concentrated our correlation analysis on four immune cell subsets with established roles in RA pathogenesis: neutrophils, macrophages, dendritic cells (activated and resting), and NK cells.. Testing all 22 cell types against eight hub genes would have yielded 176 correlations, the vast majority of which are physiologically meaningless. We computed Spearman correlation coefficients for hub gene expression levels and immune cell numbers in these groups. Correlations having $|r| > 0.45$ and $p < 0.05$ were displayed in a heatmap utilizing the corrplotR software.

3.7. External Transcriptomics Validation

3.7.1. Gene expression analysis

The external validation cohort was selected and the independent dataset GSE55457 was utilized to confirm the expression values of hub genes. The study comprised 13 RA patients and 10 controls, along with synovial samples (152). To minimize variations between different cohorts, appropriate data normalization and batch effect adjustment methods were applied. Differential

gene expression analysis was subsequently conducted using the limma package in the R programming environment, while multiple comparison corrections were carried out using the Benjamini–Hochberg procedure to maintain control over the false discovery rate (FDR) (153). The eight hub genes were chosen, and their expression was evaluated across the samples to determine their differential expression between RA and controls. Genes with adjusted $p < 0.05$ and comparable expression patterns to the discovery group were verified. Plots were created with ggplot2 and pheatmap.

3.7.2. ROC curve analysis:

For evaluation of diagnostic performance for primary targets, we conducted Receiver Operating Characteristic (ROC) curve assessment with independent dataset validation using GSE55457 and the pROC package in R (154). The expression levels of each hub gene were used as predictor variables, and disease status (rheumatoid arthritis vs control) being the binary outcome. Area under curve (AUC) having a confidence interval of 95% was determined over 2000 stratified bootstrapping rounds. An AUC value of more than 0.70 showed adequate diagnostic performance.

3.8. ADMET Analysis

The pharmacokinetics and toxicity of columbin were anticipated using multiple in silico ADMET prediction techniques. Physicochemical parameters were predicted using SwissADME, whereas permeability, metabolism, and toxicity endpoints were predicted with pkCSM (155,156). ProTox 3.0 predicted additional toxicity indicators, including as hepatotoxicity, drug-induced liver damage (DILI), and carcinogenicity. 0 (157).

CHAPTER - 4

RESULT AND DISCUSSION

4.1. Columbin and Disease Targets Identification

A total of 169 possible columbin targets were identified using SwissTargetPrediction, PharmMapper, TargetNet, and SuperPred. In parallel, 3,037 inflammation-related genes were discovered using GSE55235, DisGeNET, and GeneCards. Differential expression genes were analyzed using the GEO dataset, and significant up- and down-regulated genes were identified and shown using the volcano plot (fig. 2A). Venn diagram analysis revealed 107 genes at the intersection of columbin predicted targets (n=169) and inflammation-related genes (n=3,037), accounting for 63.3% of columbin targets with known inflammatory correlations (fig. 2B). These 107 genes underwent PPI network analysis.

4.2. PPI Network and Hub Gene Identification

Among the discovered genes, 90 (84.1%) were successfully mapped through STRING database, resulting in linked PPI network. The remaining 17 genes had no interactions at the chosen high-confidence level (≥ 0.7) and were thus eliminated from further network analysis. Cytoscape displayed the PPI network (figs. 3A,3B), and the structure was investigated. The top regulatory genes in PPI network were identified using 4 methods from CytoHubba plugin to analyze their topological structure. CytoHubba identified eight core targets that rated in the top ten for all four centrality criteria (Degree, Betweenness, Closeness, and MCC). The following genes (ESR1, JAK2, PIK3R1, STAT3, HSP90AA1, JAK1, MAPK1, and PTPN11) were chosen for molecular docking and validation (Fig. 3B).

4.3. Functional Enrichment Analysis

DAVID functional enrichment study of overlapping genes revealed that the enhanced biological activities are mostly related to signal transmission, transcription, and cell proliferation. A Gene Ontology (GO) study identified 69 substantially enriched biological processes (FDR < 0.05) related to stimulus response and cell proliferation. The top keywords are "signal transduction" (n = 19 genes) and "positive regulation of transcription by RNA polymerase II" (n = 17 genes) (Figure 4A). Analysis of cellular components (CC) revealed high enrichment in cytoplasm (47 genes) and cytosol (40 genes), consistent with role of targets in intracellular signaling pathways. Molecular function (MF) study found a high enrichment in protein binding (60 genes) and metal ion binding (34 genes).

KEGG pathway enrichment study recognized 57 highly enriched pathways ($FDR < 5E-02$), primarily associated with cancer processes and signal transduction. The most significantly enriched pathways were "Pathways in cancer" ($n = 26$ genes), "MicroRNAs in cancer" ($n = 12$ genes), and "PI3K-Akt signalling pathway" ($n = 12$ genes) (Figure 4B). These findings demonstrate the importance of the identified targets in cancer control and key signal transduction pathways.

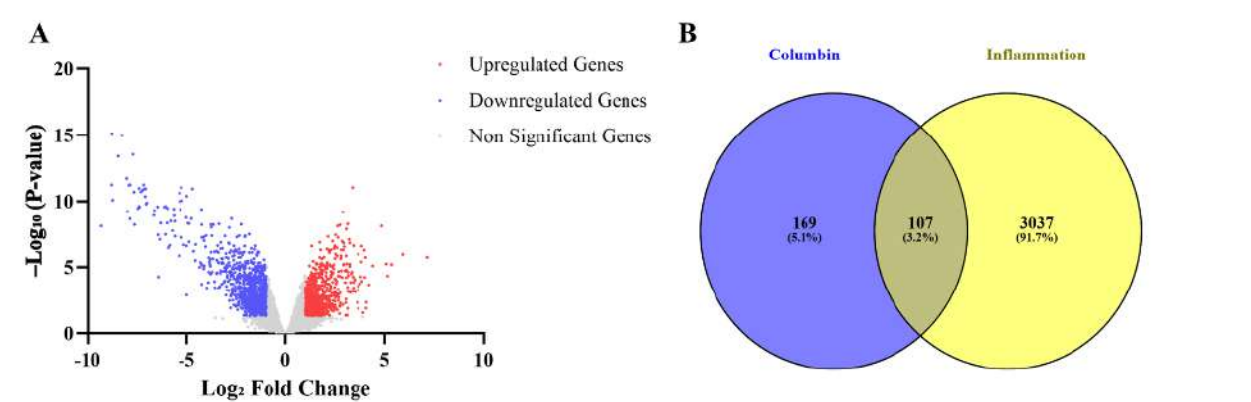


Fig. 2: --- Network building and gene identification. (A) A volcano map of DEGs from GSE55235. (B) Venn diagram depicting the overlapping targets of columbin and inflammation-associated genes.

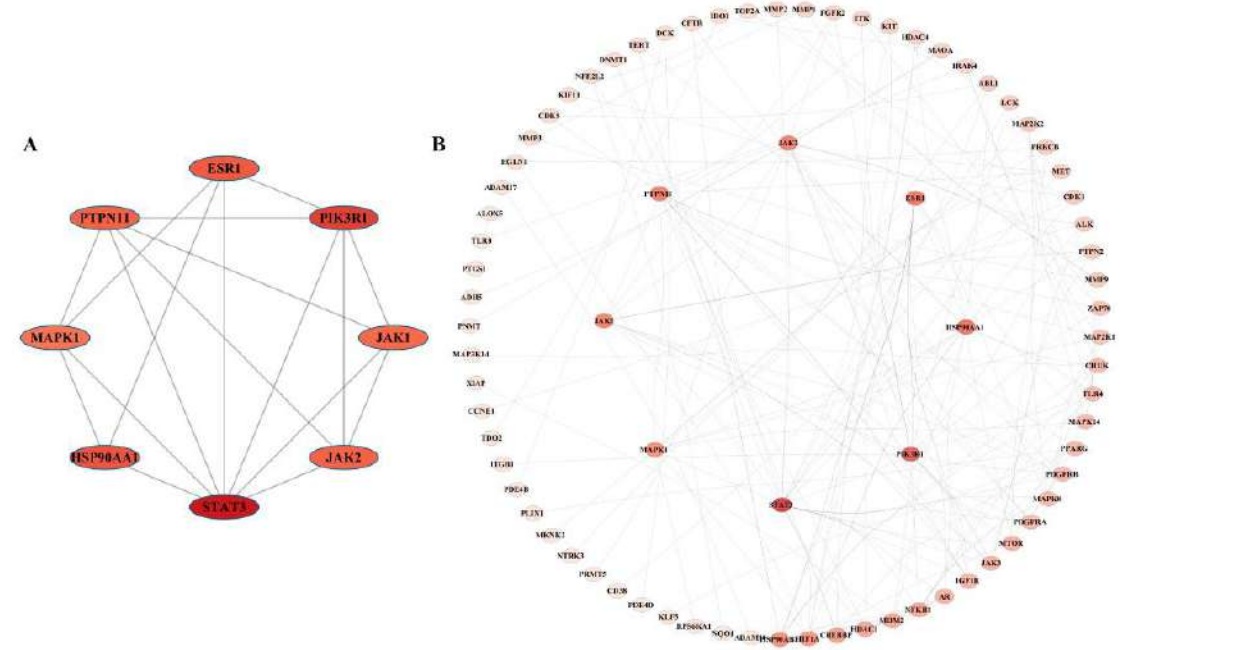


Fig. 3: --- (A) PPI network with overlapping targets; (B) CytoHubba-identified hub gene network.

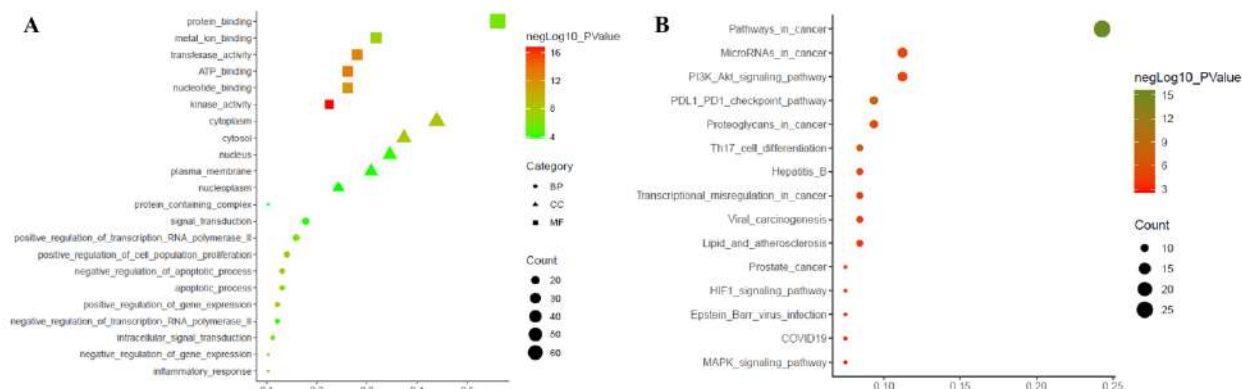


Fig. 4: --- (A) GO enrichment analysis identifies BP, CC, and MF. (B) The KEGG pathway study reveals shared targets.

4.4. Molecular Docking Analysis

Columbin showed good binding for every targets, including auto-dock binding energies spanning -6.9 to -8.6 kcal/mol as well as Swiss Dock binding energy varying from -6.4 to -7.7 kcal/mol. Most effective targets are ESR1 (-8.6 kcal/mol), HSP90AA1 (-8.4 kcal/mol), and MAPK1 (-8.3 kcal/mol) (Table 1). When compared to native ligands, it is clear that, although having somewhat lower binding energies, columbin persistently bound to the active binding site. A comprehensive investigation of columbin's interaction with targets revealed that it formed several hydrogen bonds having active site residues (fig. 5). Hydrogen bond interactions ranged from 1.87 to 3.42 Å. All docked complexes had RMSD values below 2 Å, indicating columbin's stable binding interactions.

Table 1 – Molecular docking results of columbin and native ligand with core targets

Protein	PDB IDs	Autodock ΔG (kcal/mol)	SwissDock ΔG (kcal/mol)	Native Ligand	Native ligand binding energy (kcal/mol)	Residue involved in H bonding	H-bond length (Å)	RM SD
ESR1	1A52	-8.615	-6.7564	EST	-10.75	ARG394, LEU525, GLU353	2.63, 3.09, 2.92	0.469
JAK2	2B7A	-7.306	-6.7427	IZA	-11.11	ASP939, LYS857	2.64, 2.22	0.893
PIK3CA-PIK3R1	7PG6	-7.892	-6.9448	1LT	-10.02	TYR836, VAL851, ASP933, GLU849	2.46, 1.94, 2.25, 3.29	1.65
STAT3	6NJS	-7.074	-7.663	KQV	-7.221	ILE653, TYR640, GLU638, TYR657, VAL637	1.87, 1.92, 2.83, 2.63, 2.79	1.851
HSP90AA1	3O0I	-8.4	-7.2709	P54	-9.564	ASN51	2.25	1.774
JAK1	3EYG	-6.957	-6.4467	MI1	-7.86	HIS885, ASN1008	2.94, 3.42	0.402
MAPK1	1WZY	-8.3	-7.6822	F29	-8.243	LYS54, GLU109,	2.86, 1.96,	1.25
PTPN11	3B7O	-8.072	-7.7772	MLT	-4.814	GLU361, SER460, ALA461, ARG362	3.01, 3.02, 2.81, 3.35	1.583

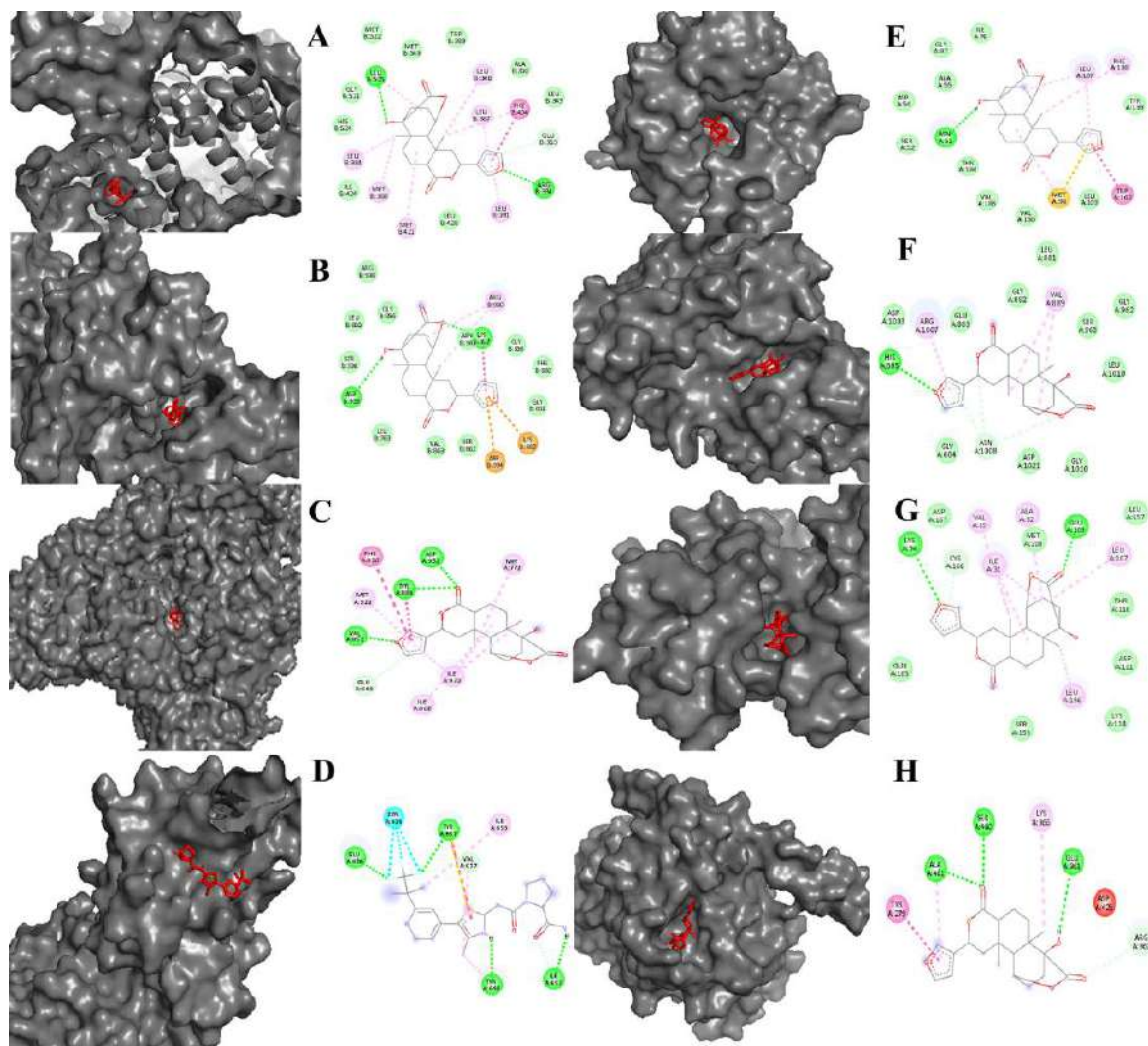


Fig. 5: ---3D visualization of the docked ligand with target protein, showing binding pocket interactions and ligand orientation obtained from molecular docking studies – A-ESR1, B-JAK2, C-PIK3CA-PIK3R1, D- STAT3, E-HSP90AA1, F-JAK1, G-MAPK1 and H-PTPN11

4.5. Immune cell infiltration analysis

To further understand how hub genes influence immunological responses in rheumatoid arthritis, we looked at connections between gene expression and immune cell infiltration. Rather of evaluating all 22 cell types, which would result in more false positives, we concentrated on physiologically important populations known to cause RA pathology: neutrophils, macrophages, dendritic cells, and NK cells. Several hub genes were strongly associated with particular immunological populations (figure 6). MAPK1 substantially correlates with neutrophils ($r=0.69$, $p<0.001$), supporting its function in neutrophil activation and chemotaxis. JAK2 was also related

with neutrophils ($r=0.49$, $p=0.03$), which is consistent with JAK-STAT signaling in myeloid cell activity (fig. 6).

HSP90AA1 has an unfavorable connection with neutrophils ($r=-0.55$, $p=0.01$), indicating that it may reduce rather than increase inflammatory responses. Resting NK cells revealed the largest negative correlations: PIK3R1 and PTPN11, presumably representing NK cell activation or redistribution during inflammation. STAT3, ESR1, and JAK1 exhibited lesser or non-significant associations with these cell types, indicating that their activities may be context-dependent or include other immune populations not studied here. These findings suggest that hub genes interact differentially with the immunological microenvironment: some promote neutrophil-induced inflammation (MAPK1, JAK2), while others may control innate immunity via NK cell pathways (PIK3R1, PTPN11).

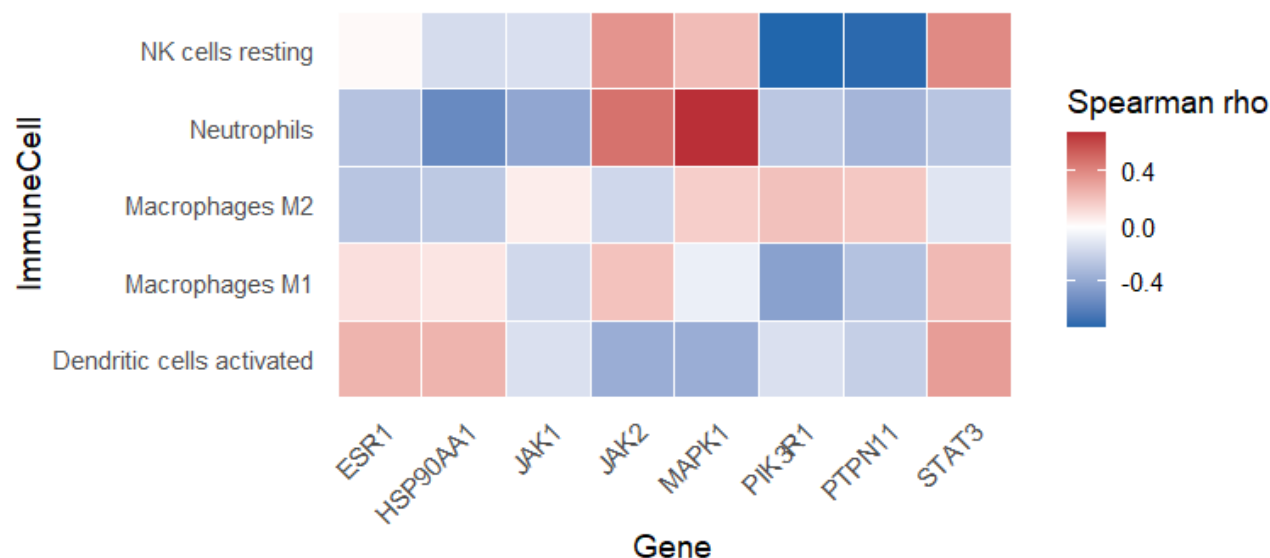


Fig. 6: --- CIBERSORT-based heatmap showing the relative proportions of immune cell infiltration across samples

4.6. Bioinformatics Validation

4.6.1. Gene Expression Analysis

In the validation cohort (GSE55457, $n=13$ RA versus $n=10$ controls), two out of eight hub genes (JAK2 and HSP90AA1) were substantially differently expressed (adjusted $p<0.05$) (fig. 7A). Despite small fold variations (0.6 to 1.8-fold), hierarchical clustering using these 8 genes efficiently distinguished RA and control samples (fig. 7B), indicating that coordinated expression alterations across several genes contribute to disease pathophysiology. These findings indicate a

network-based mechanism in which columbin targets serve as regulatory nodes rather than severely dysregulated effectors. The diagnostic usefulness exhibited by ROC analysis despite minor expression variations shows that these genes display consistent directional changes that collectively differentiate disease states.

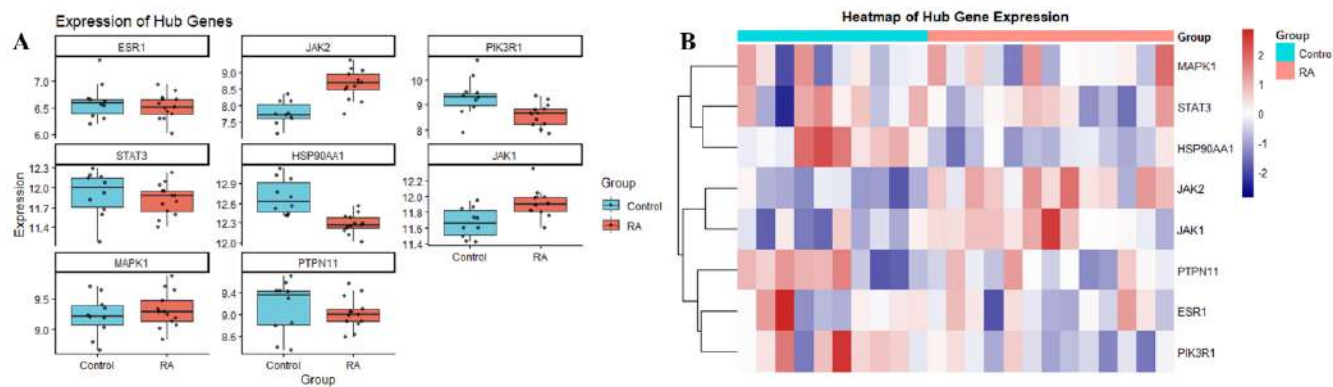


Fig. 7: --- (A) Boxplot showing DEGs in the GEO dataset between disease and control samples. (B) Heatmap depicting expression patterns of DEGs between samples in the GEO dataset.

4.7.2. ROC Curve Analysis

ROC curve research found that 5 of 8 hub genes had an AUC greater than 0.70, indicating acceptable to exceptional discriminating capacity between RA and control samples (Fig. 8, Table 2). The high AUC values achieved by JAK1, JAK2, and HSP90AA1 confirmed the potential for genes to further be used as diagnostic biomarkers to distinguish RA from controls, whereas ESR1, PTPN11, and MAPK1 have a poor predictive value among all core target genes, implying that its role in inflammation is more regulatory or context-dependent instead of consistently dysregulated in all RA patients.

Table 2 --- AUC values from ROC analysis representing the diagnostic performance of core targets.

Gene	LogFC	Average Expression	t-statistic	P.Value	Adjusted P-value	B-statistic	FoldChange
JAK2	0.8593	8.2601	5.0034	4.23E-05	0.0053	2.1921	1.8142
JAK1	0.2468	11.8005	2.5915	1.61E-02	0.1073	-3.3007	1.1866
MAPK1	0.0914	9.2762	0.6668	5.11E-01	0.7281	-6.0362	1.0654
STAT3	-0.0804	11.8559	-0.6157	5.44E-01	0.7489	-6.0685	0.9458
PTPN11	-0.1022	9.0631	-0.6190	5.42E-01	0.7474	-6.0665	0.9316

ESR1	-0.1028	6.5653	-0.7868	4.39E-01	0.6741	-5.9506	0.9312
HSP90AA1	-0.4080	12.4630	-3.9637	5.86E-04	0.0186	-0.2642	0.7537
PIK3R1	-0.7334	8.9157	-2.8821	8.24E-03	0.0744	-2.7002	0.6015

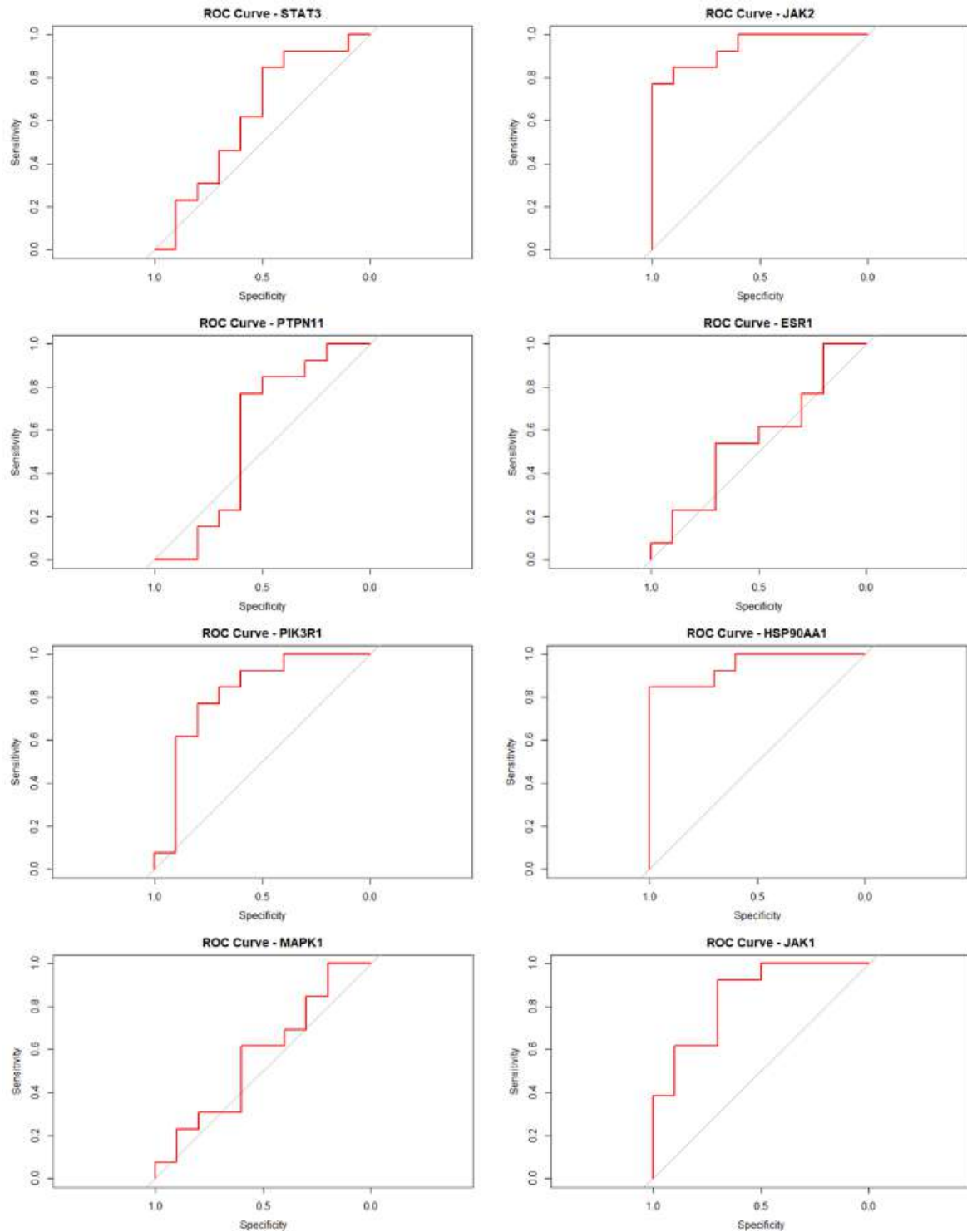


Fig. 8: --- ROC curve depicting the diagnostic performance of key genes with corresponding AUC values.

4.7. ADMET Analysis

Columbin has good pharmacokinetic features, including good intestinal absorption, permeability in the Caco-2 system (0.986), and great human intestine absorption (97.48%), indicating good oral bioavailability (table 3). The physicochemical properties showed moderate lipophilicity (LogP = 2.13) and adequate solubility (LogS = -3.5), with no Lipinski rule violations, indicating its drug-likeness. According to metabolism projections, columbin is a CYP3A4 substrate but does not inhibit key CYP isoforms (CYP3A4, 2D6, 2C9, 1A2), indicating a limited possibility for drug-drug interactions. ADMET projected no carcinogenic potential (AMES negative), low hERG inhibition risk ($IC_{50} > 10 \mu M$ expected), and low skin sensitization likelihood (0.12). However, worrisome toxicity signals were indicated, including a high DILI likelihood (0.85) and carcinogenicity potential (0.62), which require experimental confirmation (Table 3).

The ADMET analysis of columbin (fig. 9) demonstrates that the molecule has high GI absorption because it is within the white area in the BOILED-Egg plot, suggesting good intestinal permeability. But columbin does not appear in the yolk region, indicating that it is not likely to cross BBB. Bioavailability radar enhances acceptable drug-likeness qualities in the optimal physicochemical space.

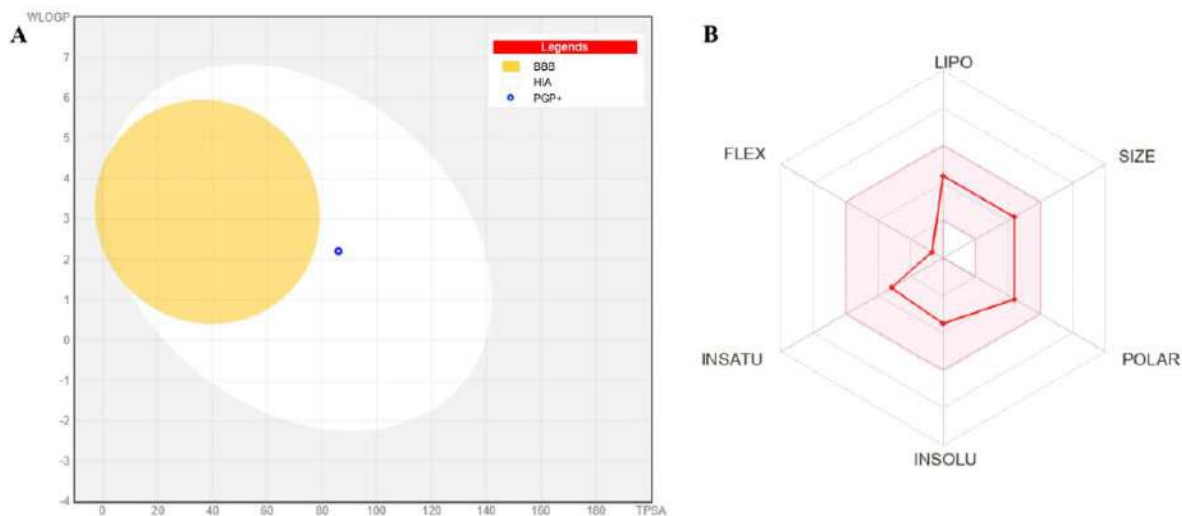


Fig. 9: ((A) The BOILED-Egg model analysis illustrates the predicted intestinal uptake and potential ability of columbin to penetrate the BBB, determined using WLOGP and topological polar surface area (TPSA) parameters. In the plot, columbin is represented by a blue marker. (B) The bioavailability radar profile of columbin highlights its physicochemical

and pharmacokinetic characteristics associated with drug-likeness, such as molecular size, lipid affinity, polarity, aqueous solubility, structural flexibility, and degree of saturation.

Table 3: --- ADMET properties of columbin predicted using in silico tools

Pharmacokinetic Class	Property Evaluated	Observed Outcome	Drug Profile Class	Property Evaluated	Observed Outcome
Absorption	Gastrointestinal Uptake	High absorption predicted	Drug-Likeness	Lipinski's Rule Assessment	No rule violations detected
	Caco-2 Cell Permeability (log Papp, 10 ⁻⁶ cm/s)	0.986		Molecular Mass (Da)	358.39
	Predicted Human Intestinal Absorption (%)	97.485		Partition Coefficient (Log P)	2.13
	P-glycoprotein (P-gp) Substrate Status	Non-substrate		Hydrogen Bond Donors	1
	P-glycoprotein (P-gp) Inhibitory Activity	No inhibitory effect		Hydrogen Bond Acceptors	6
	Aqueous Solubility (log S)	-3.48		Topological Polar Surface Area (TPSA, Å ²)	85.97
Distribution	Blood-Brain Barrier Penetration (log BB)	-0.284		Number of Rotatable Bonds	1
	Volume of Distribution (log L/kg)	0.137		Predicted Bioavailability Score	0.55

	Fraction of Unbound Compound (Fu)	0.205		Synthetic Accessibility Index	5.37
Metabolism	CYP3A4 Metabolic Substrate	Yes	Toxicological Profile	AMES Mutagenicity Test	Negative
	CYP3A4 Inhibitory Potential	Not detected		Hepatotoxic Potential	Positive
	CYP2D6 Inhibition	No inhibitory activity		hERG Channel Inhibition	Absent
	CYP2C9 Inhibition	Negative		Median Lethal Dose (LD ₅₀ , mg/kg)	2.946
	CYP1A2 Inhibition	Negative		Skin Sensitization Risk	Not predicted
Excretion	Total Body Clearance (log ml/min/kg)	0.79		Drug-Induced Liver Injury (DILI) Score	0.85
	Renal OCT2 Transporter Substrate	Yes		Carcinogenicity Probability	0.62

CHAPTER - 6

CONCLUSION AND FUTURE PERSPECTIVE

Columbin's multi-targeted anti-inflammatory mechanism was explained in detail utilizing a comprehensive computational framework that included network pharmacology, molecular docking, transcriptome validation, interactions between proteins, and immune infiltration profiling, and ADMET prediction. Using target prediction, disease-associated gene sets, and transcriptomics data, the researchers identified numerous physiologically relevant hub targets implicated in chronic inflammatory signaling. The findings show that, despite columbin's low molecular weight and structurally simple phytochemical architecture, it has a high binding affinity for several inflammatory proteins, implying its potential as a multi-target therapeutic scaffold capable of modulating interconnected inflammatory pathways rather than a single molecular mechanism.

The identified hub genes, including ESR1, JAK1, JAK2, MAPK1, STAT3, HSP90AA1, PIK3R1 and PTPN11, were shown to be involved in important inflammatory signalling pathways, all of them having crucial effect on development and maintenance of chronic inflammatory disorders. Interactions were validated by molecular docking analysis and showed favourable binding energies, interaction profiles, and docking protocol validation by co-crystal ligand redocking, further supporting the stability and specificity of these interactions. Moreover, several of the targets identified were shown to be relevant to rheumatoid arthritis using independent disease datasets of transcripts, suggesting their diagnostic relevance. Immune infiltration analysis also found critical associations between gene expression of hub genes specifically neutrophils and macrophages, further supporting the immunomodulatory potential of the predicted targets. Additionally, the study emphasises the significance and advancement of network pharmacology methods in modern phytochemical drug discovery. The current paradigm is different from the previous paradigm of single target-based drug discovery in that it emphasizes complexity of inflammatory diseases and the importance of understanding process crosstalk and target interconnectivity, in addition to multi-level control of biological processes. This computational workflow set up in this investigation can hence be used as a powerful and repeatable tool for network-based screening and mechanistic characterization of other therapeutic potential bioactive natural products. While the results strongly indicate anti-inflammatory effects for columbin, there are a number

of caveats and future directions that should be explored. Experimental validation of the predicted targets and pathways using biochemical binding assays, gene expression and cell-based inflammatory models should be explored in the future. Structure-activity relationship (SAR) studies and rational analogue design should also be considered to maximize the binding potency, binding selectivity and pharmacokinetic behaviour while minimizing the predicted hepatotoxicity found during ADMET evaluation. Additionally, molecular dynamic simulations and free-energy calculations may provide greater insight into steadiness and conformational behaviour of protein-ligand complexes under physiological settings. Finally, in vivo preclinical research of columbin as prospective of multi target as well as anti-inflammatory candidate will be required in relevant inflammatory disease models to assess its therapeutic potential, safety profile, and translatability.

CHAPTER - 7

REFERENCES

1. Furman D, Campisi J, Verdin E, Carrera-Bastos P, Targ S, Franceschi C, et al. Chronic inflammation in the etiology of disease across the life span. *Nat Med*. 2019 Dec;25(12):1822–32. doi:10.1038/s41591-019-0675-0
2. Pahwa R, Goyal A, Jialal I. Chronic Inflammation. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2026 [cited 2026 Apr 15]. Available from: <http://www.ncbi.nlm.nih.gov/books/NBK493173/> PubMed PMID: 29630225.
3. Sostres C, Gargallo CJ, Arroyo MT, Lanás A. Adverse effects of non-steroidal anti-inflammatory drugs (NSAIDs, aspirin and coxibs) on upper gastrointestinal tract. *Best Pract Res Clin Gastroenterol*. 2010 Apr;24(2):121–32. doi:10.1016/j.bpg.2009.11.005 PubMed PMID: 20227026.
4. Buchman AL. Side Effects of Corticosteroid Therapy. *J Clin Gastroenterol*. 2001 Oct;33(4):289.
5. Puca P, Capobianco I, Coppola G, Di Vincenzo F, Trapani V, Petito V, et al. Cellular and Molecular Determinants of Biologic Drugs Resistance and Therapeutic Failure in Inflammatory Bowel Disease. *Int J Mol Sci*. 2024 Jan;25(5):2789. doi:10.3390/ijms25052789
6. Murray PJ, Smale ST. Restraint of inflammatory signaling by interdependent strata of negative regulatory pathways. *Nat Immunol*. 2012 Oct;13(10):916–24. doi:10.1038/ni.2391 PubMed PMID: 22990889; PubMed Central PMCID: PMC4893774.
7. Ge Y jun, Liao Q wen, Xu Y chun, Zhao Q, Wu B li, Ye RD. Anti-inflammatory signaling through G protein-coupled receptors. *Acta Pharmacol Sin*. 2020 Dec;41(12):1531–8. doi:10.1038/s41401-020-00523-1 PubMed PMID: 33060777; PubMed Central PMCID: PMC7921558.
8. Traditional and clinical use of *Tinospora cordifolia*, guduchi. *Aust J Med Herbal* [Internet]. [cited 2026 Apr 15]. Available from: <https://search.informit.org/doi/abs/10.3316/informit.408609094525223>
9. Jo M, Va R, Jd C, Pj H. Anti-inflammatory activities of the methanol extracts and an isolated furanoditerpene constituent of *Sphenocentrum jollyanum* Pierre (Menispermaceae). *J Ethnopharmacol*. 2006 Mar 8;104(1–2). doi:10.1016/j.jep.2005.08.051 PubMed PMID: 16236477.

10. Ibrahim Abdelwahab S, Syaed Koko W, Mohamed Elhassan Taha M, Mohan S, Achoui M, Ameen Abdulla M, et al. *In vitro* and *in vivo* anti-inflammatory activities of columbin through the inhibition of cyclooxygenase-2 and nitric oxide but not the suppression of NF- κ B translocation. *Eur J Pharmacol.* 2012 Mar 5;678(1):61–70. doi:10.1016/j.ejphar.2011.12.024
11. Dash S, Verma S. Phytopharmacological profile of *Tinospora cordifolia*: An updated review. *J Pharmacogn Phytochem.* 2026 Mar 9;15(2):47–55. doi:10.22271/phyto.2026.v15.i2a.15770
12. Ibrahim Abdelwahab S, Syaed Koko W, Mohamed Elhassan Taha M, Mohan S, Achoui M, Ameen Abdulla M, et al. In vitro and in vivo anti-inflammatory activities of columbin through the inhibition of cyclooxygenase-2 and nitric oxide but not the suppression of NF- κ B translocation. *Eur J Pharmacol.* 2012 Mar 5;678(1–3):61–70. doi:10.1016/j.ejphar.2011.12.024 PubMed PMID: 22227329.
13. Walker KA, Le Page LM, Terrando N, Duggan MR, Heneka MT, Bettcher BM. The role of peripheral inflammatory insults in Alzheimer’s disease: a review and research roadmap. *Mol Neurodegener.* 2023 Jun 5;18(1):37. doi:10.1186/s13024-023-00627-2 PubMed PMID: 37277738; PubMed Central PMCID: PMC10240487.
14. Cifuentes M, Verdejo HE, Castro PF, Corvalan AH, Ferreccio C, Quest AFG, et al. Low-Grade Chronic Inflammation: a Shared Mechanism for Chronic Diseases. *Physiology.* 2025 Jan 1;40(1):0. doi:10.1152/physiol.00021.2024 PubMed PMID: 39078396.
15. Pawelec G, Goldeck D, Derhovanessian E. Inflammation, ageing and chronic disease. *Curr Opin Immunol.* 2014 Aug;29:23–8. doi:10.1016/j.coi.2014.03.007 PubMed PMID: 24762450.
16. Evavold CL, Kagan JC. How Inflammasomes Inform Adaptive Immunity. *J Mol Biol.* 2018 Jan 19;430(2):217–37. doi:10.1016/j.jmb.2017.09.019 PubMed PMID: 28987733; PubMed Central PMCID: PMC5766381.
17. Ortega-Zapero M, Gomez-Bris R, Pascual-Laguna I, Saez A, Gonzalez-Granado JM. Neutrophils and NETs in Pathophysiology and Treatment of Inflammatory Bowel Disease. *Int J Mol Sci.* 2025 Jul 23;26(15):7098. doi:10.3390/ijms26157098 PubMed PMID: 40806230; PubMed Central PMCID: PMC12346662.
18. Kinney JW, Bemiller SM, Murtishaw AS, Leisgang AM, Salazar AM, Lamb BT. Inflammation as a central mechanism in Alzheimer’s disease. *Alzheimers Dement Transl Res Clin Interv.* 2018 Sep 6;4:575–90. doi:10.1016/j.trci.2018.06.014 PubMed PMID: 30406177; PubMed Central PMCID: PMC6214864.

19. Kany S, Vollrath JT, Relja B. Cytokines in Inflammatory Disease. *Int J Mol Sci.* 2019 Nov 28;20(23):6008. doi:10.3390/ijms20236008 PubMed PMID: 31795299; PubMed Central PMCID: PMC6929211.
20. Ishijima T, Nakajima K. Inflammatory cytokines TNF α , IL-1 β , and IL-6 are induced in endotoxin- stimulated microglia through different signaling cascades. *Sci Prog.* 2021 Oct;104(4):368504211054985. doi:10.1177/00368504211054985 PubMed PMID: 34821182; PubMed Central PMCID: PMC10450609.
21. Navegantes KC, de Souza Gomes R, Pereira PAT, Czaikoski PG, Azevedo CHM, Monteiro MC. Immune modulation of some autoimmune diseases: the critical role of macrophages and neutrophils in the innate and adaptive immunity. *J Transl Med.* 2017 Feb 15;15(1):36. doi:10.1186/s12967-017-1141-8 PubMed PMID: 28202039; PubMed Central PMCID: PMC5312441.
22. Hirano T. IL-6 in inflammation, autoimmunity and cancer. *Int Immunol.* 2021 Mar 1;33(3):127–48. doi:10.1093/intimm/dxaa078 PubMed PMID: 33337480; PubMed Central PMCID: PMC7799025.
23. Hughes CE, Nibbs RJB. A guide to chemokines and their receptors. *FEBS J.* 2018 Aug;285(16):2944–71. doi:10.1111/febs.14466 PubMed PMID: 29637711; PubMed Central PMCID: PMC6120486.
24. Bhusal RP, Foster SR, Stone MJ. Structural basis of chemokine and receptor interactions: Key regulators of leukocyte recruitment in inflammatory responses. *Protein Sci Publ Protein Soc.* 2020 Feb;29(2):420–32. doi:10.1002/pro.3744 PubMed PMID: 31605402; PubMed Central PMCID: PMC6954698.
25. Eckardt V, Miller MC, Blanchet X, Duan R, Leberzammer J, Duchene J, et al. Chemokines and galectins form heterodimers to modulate inflammation. *EMBO Rep.* 2020 Apr 3;21(4):e47852. doi:10.15252/embr.201947852 PubMed PMID: 32080959; PubMed Central PMCID: PMC7132340.
26. Abdulkhaleq LA, Assi MA, Abdullah R, Zamri-Saad M, Taufiq-Yap YH, Hezmee MNM. The crucial roles of inflammatory mediators in inflammation: A review. *Vet World.* 2018 May;11(5):627–35. doi:10.14202/vetworld.2018.627-635 PubMed PMID: 29915501; PubMed Central PMCID: PMC5993766.

27. Austermann J, Roth J, Barczyk-Kahlert K. The Good and the Bad: Monocytes' and Macrophages' Diverse Functions in Inflammation. *Cells*. 2022 Jun 20;11(12):1979. doi:10.3390/cells11121979 PubMed PMID: 35741108; PubMed Central PMCID: PMC9222172.
28. Zhao H, Wu L, Yan G, Chen Y, Zhou M, Wu Y, et al. Inflammation and tumor progression: signaling pathways and targeted intervention. *Signal Transduct Target Ther*. 2021 Jul 12;6(1):263. doi:10.1038/s41392-021-00658-5 PubMed PMID: 34248142; PubMed Central PMCID: PMC8273155.
29. Prame Kumar K, Nicholls AJ, Wong CHY. Partners in crime: neutrophils and monocytes/macrophages in inflammation and disease. *Cell Tissue Res*. 2018 Mar;371(3):551–65. doi:10.1007/s00441-017-2753-2 PubMed PMID: 29387942; PubMed Central PMCID: PMC5820413.
30. Sun SC. The non-canonical NF- κ B pathway in immunity and inflammation. *Nat Rev Immunol*. 2017 Sep;17(9):545–58. doi:10.1038/nri.2017.52 PubMed PMID: 28580957; PubMed Central PMCID: PMC5753586.
31. Wong MM, Fish EN. Chemokines: attractive mediators of the immune response. *Semin Immunol*. 2003 Feb;15(1):5–14. doi:10.1016/S1044-5323(02)00123-9
32. Yeung YT, Aziz F, Guerrero-Castilla A, Arguelles S. Signaling Pathways in Inflammation and Anti-inflammatory Therapies. *Curr Pharm Des*. 2018;24(14):1449–84. doi:10.2174/1381612824666180327165604 PubMed PMID: 29589535.
33. Zhang T, Ma C, Zhang Z, Zhang H, Hu H. NF- κ B signaling in inflammation and cancer. *MedComm*. 2021 Dec;2(4):618–53. doi:10.1002/mco2.104 PubMed PMID: 34977871; PubMed Central PMCID: PMC8706767.
34. Yu H, Lin L, Zhang Z, Zhang H, Hu H. Targeting NF- κ B pathway for the therapy of diseases: mechanism and clinical study. *Signal Transduct Target Ther*. 2020 Sep 21;5(1):209. doi:10.1038/s41392-020-00312-6 PubMed PMID: 32958760; PubMed Central PMCID: PMC7506548.
35. Agrawal N. A Comprehensive Review on the Advancements of Dual COX-2/5-LOX Inhibitors as Anti-Inflammatory Drugs. *Chem Biol Drug Des*. 2025 May;105(5):e70114. doi:10.1111/cbdd.70114 PubMed PMID: 40346930.

36. Mussbacher M, Derler M, Basilio J, Schmid JA. NF- κ B in monocytes and macrophages – an inflammatory master regulator in multitasked immune cells. *Front Immunol*. 2023 Feb 23;14. doi:10.3389/fimmu.2023.1134661
37. Haftcheshmeh SM, Abedi M, Mashayekhi K, Mousavi MJ, Navashenaq JG, Mohammadi A, et al. Berberine as a natural modulator of inflammatory signaling pathways in the immune system: Focus on NF- κ B, JAK/STAT, and MAPK signaling pathways. *Phytother Res PTR*. 2022 Mar;36(3):1216–30. doi:10.1002/ptr.7407 PubMed PMID: 35142403.
38. Chen Y, Fang ZM, Yi X, Wei X, Jiang DS. The interaction between ferroptosis and inflammatory signaling pathways. *Cell Death Dis*. 2023 Mar 21;14(3):205. doi:10.1038/s41419-023-05716-0
39. Hu Q, Bian Q, Rong D, Wang L, Song J, Huang HS, et al. JAK/STAT pathway: Extracellular signals, diseases, immunity, and therapeutic regimens. *Front Bioeng Biotechnol*. 2023 Feb 23;11:1110765. doi:10.3389/fbioe.2023.1110765 PubMed PMID: 36911202; PubMed Central PMCID: PMC9995824.
40. Xin P, Xu X, Deng C, Liu S, Wang Y, Zhou X, et al. The role of JAK/STAT signaling pathway and its inhibitors in diseases. *Int Immunopharmacol*. 2020 Mar;80:106210. doi:10.1016/j.intimp.2020.106210 PubMed PMID: 31972425.
41. Hu X, Li J, Fu M, Zhao X, Wang W. The JAK/STAT signaling pathway: from bench to clinic. *Signal Transduct Target Ther*. 2021 Nov 26;6(1):402. doi:10.1038/s41392-021-00791-1 PubMed PMID: 34824210; PubMed Central PMCID: PMC8617206.
42. Kennedy BM, Harris RE. Cyclooxygenase and Lipoxygenase Gene Expression in the Inflammogenesis of Colorectal Cancer: Correlated Expression of EGFR, JAK STAT and Src Genes, and a Natural Antisense Transcript, RP11-C67.2.2. *Cancers*. 2023 Apr 20;15(8):2380. doi:10.3390/cancers15082380 PubMed PMID: 37190308; PubMed Central PMCID: PMC10136816.
43. Brune K, Patrignani P. New insights into the use of currently available non-steroidal anti-inflammatory drugs. *J Pain Res*. 2015;8:105–18. doi:10.2147/JPR.S75160 PubMed PMID: 25759598; PubMed Central PMCID: PMC4346004.
44. Ferrer MD, Busquets-Cortés C, Capó X, Tejada S, Tur JA, Pons A, et al. Cyclooxygenase-2 Inhibitors as a Therapeutic Target in Inflammatory Diseases. *Curr Med Chem*.

- 2019;26(18):3225–41. doi:10.2174/0929867325666180514112124 PubMed PMID: 29756563.
45. Pereira-Leite C, Nunes C, Jamal SK, Cuccovia IM, Reis S. Nonsteroidal Anti-Inflammatory Therapy: A Journey Toward Safety. *Med Res Rev.* 2017 Jul;37(4):802–59. doi:10.1002/med.21424 PubMed PMID: 28005273.
 46. Moore N, Duong M, Gulmez SE, Blin P, Droz C. Pharmacoepidemiology of non-steroidal anti-inflammatory drugs. *Therapie.* 2019 Apr;74(2):271–7. doi:10.1016/j.therap.2018.11.002 PubMed PMID: 30477749.
 47. Rao P, Knaus EE. Evolution of nonsteroidal anti-inflammatory drugs (NSAIDs): cyclooxygenase (COX) inhibition and beyond. *J Pharm Pharm Sci Publ Can Soc Pharm Sci Soc Can Sci Pharm.* 2008 Sep 20;11(2):81s–110s. doi:10.18433/j3t886 PubMed PMID: 19203472.
 48. Stiller CO, Hjemdahl P. Lessons from 20 years with COX-2 inhibitors: Importance of dose-response considerations and fair play in comparative trials. *J Intern Med.* 2022 Oct;292(4):557–74. doi:10.1111/joim.13505 PubMed PMID: 35585779.
 49. Meretskyi VM, Meretska IV. Principles of effective use of non-steroidal anti-inflammatory drugs. *Inter Collegas.* 2023 Jun 30;10(1):20–6. doi:10.35339/ic.10.1.mer
 50. Pj B. Anti-inflammatory actions of glucocorticoids: molecular mechanisms. *Clin Sci Lond Engl* 1979. 1998 Jun;94(6). doi:10.1042/cs0940557 PubMed PMID: 9854452.
 51. Goppelt-Struebe M, Wolter D, Resch K. Glucocorticoids inhibit prostaglandin synthesis not only at the level of phospholipase A2 but also at the level of cyclo-oxygenase/PGE isomerase. *Br J Pharmacol.* 1989 Dec;98(4):1287. doi:10.1111/j.1476-5381.1989.tb12676.x PubMed PMID: 2514948.
 52. Li X, Lau SK, Woo PC. Fungal infection risks associated with the use of cytokine antagonists and immune checkpoint inhibitors. *Exp Biol Med.* 2020 Jul 8;245(13):1104. doi:10.1177/1535370220939862 PubMed PMID: 32640893.
 53. Gurpinar E, Grizzle WE, Piazza GA. COX-Independent Mechanisms of Cancer Chemoprevention by Anti-Inflammatory Drugs. *Front Oncol.* 2013 Jul 11;3:181. doi:10.3389/fonc.2013.00181 PubMed PMID: 23875171; PubMed Central PMCID: PMC3708159.

54. Grösch S, Niederberger E, Geisslinger G. Investigational drugs targeting the prostaglandin E2 signaling pathway for the treatment of inflammatory pain. *Expert Opin Investig Drugs*. 2017 Jan;26(1):51–61. doi:10.1080/13543784.2017.1260544 PubMed PMID: 27841017.
55. Ahmadi M, Bekeschus S, Weltmann KD, Woedtke T von, Wende K. Non-steroidal anti-inflammatory drugs: recent advances in the use of synthetic COX-2 inhibitors. *RSC Med Chem*. 2022 May 25;13(5):471–96. doi:10.1039/D1MD00280E
56. El-Saadony MT, Saad AM, Mohammed DM, Korma SA, Alshahrani MY, Ahmed AE, et al. Medicinal plants: bioactive compounds, biological activities, combating multidrug-resistant microorganisms, and human health benefits - a comprehensive review. *Front Immunol*. 2025 Apr 28;16:1491777. doi:10.3389/fimmu.2025.1491777 PubMed PMID: 40375989; PubMed Central PMCID: PMC12079674.
57. Nunes C dos R, Barreto Arantes M, Menezes de Faria Pereira S, Leandro da Cruz L, de Souza Passos M, Pereira de Moraes L, et al. Plants as Sources of Anti-Inflammatory Agents. *Molecules*. 2020 Jan;25(16):3726. doi:10.3390/molecules25163726
58. Nakadate K, Ito N, Kawakami K, Yamazaki N. Anti-Inflammatory Actions of Plant-Derived Compounds and Prevention of Chronic Diseases: From Molecular Mechanisms to Applications. *Int J Mol Sci*. 2025 May 28;26(11):5206. doi:10.3390/ijms26115206 PubMed PMID: 40508016; PubMed Central PMCID: PMC12154257.
59. Merecz-Sadowska A, Sitarek P, Śliwiński T, Zajdel R. Anti-Inflammatory Activity of Extracts and Pure Compounds Derived from Plants via Modulation of Signaling Pathways, Especially PI3K/AKT in Macrophages. *Int J Mol Sci*. 2020 Dec 16;21(24):9605. doi:10.3390/ijms21249605 PubMed PMID: 33339446; PubMed Central PMCID: PMC7766727.
60. Pal R, Khan A, Das S, Bharti SK, Patra A, Mandal V. Critically Analysing Plant-based Bioactives as Anti-inflammatory Agents: A molecular Phytopharmacology Perspective Coupled with Bibliometric Data. *Bioactivities*. 2025 Dec 30;3(2):96–123. doi:10.47352/bioactivities.2963-654X.337
61. Dixit K, Chauhan B, Jain R. Anti-Inflammatory Potential of Medicinal Plants in the Management of Inflammatory Diseases: A Review of Mechanisms and Bioactive Compounds. *J Drug Deliv Ther*. 2025 Jun 15;15(6):330–40. doi:10.22270/jddt.v15i6.7203

62. Mukhopadhyay N, Shukla A, Makhal PN, Kaki VR. Natural product-driven dual COX-LOX inhibitors: Overview of recent studies on the development of novel anti-inflammatory agents. *Heliyon*. 2023 Mar;9(3):e14569. doi:10.1016/j.heliyon.2023.e14569 PubMed PMID: 37020932; PubMed Central PMCID: PMC10068128.
63. Gandhi GR, Mohana T, Athesh K, Hillary VE, Vasconcelos ABS, Farias de Franca MN, et al. Anti-inflammatory natural products modulate interleukins and their related signaling markers in inflammatory bowel disease: A systematic review. *J Pharm Anal*. 2023 Dec;13(12):1408–28. doi:10.1016/j.jpha.2023.09.012 PubMed PMID: 38223446; PubMed Central PMCID: PMC10785269.
64. Nisar A, Jagtap S, Vyavahare S, Deshpande M, Harsulkar A, Ranjekar P, et al. Phytochemicals in the treatment of inflammation-associated diseases: the journey from preclinical trials to clinical practice. *Front Pharmacol*. 2023 May 9;14. doi:10.3389/fphar.2023.1177050
65. Bai R, Yao C, Zhong Z, Ge J, Bai Z, Ye X, et al. Discovery of natural anti-inflammatory alkaloids: Potential leads for the drug discovery for the treatment of inflammation. *Eur J Med Chem*. 2021 Mar 5;213:113165. doi:10.1016/j.ejmech.2021.113165 PubMed PMID: 33454546.
66. Gonfa YH, Tessema FB, Bachheti A, Rai N, Tadesse MG, Nasser Singab A, et al. Anti-inflammatory activity of phytochemicals from medicinal plants and their nanoparticles: A review. *Curr Res Biotechnol*. 2023 Jan 1;6:100152. doi:10.1016/j.crbiot.2023.100152
67. Plazas E, Sierra-Marquez L, Olivero-Verbel J. Bioactive Molecules from Tropical American Plants: Potential Anti-Inflammatory Agents for Cytokine Storm Management. *Molecules*. 2025 Jan;30(7):1486. doi:10.3390/molecules30071486
68. Subudhi L, Thatoi H, Banerjee A. Anti-inflammatory activity of essential oil from medicinal plants: An insight into molecular mechanism, in-silico studies and signaling pathways. *Phytomedicine Int J Phytother Phytopharm*. 2025 Mar;138:156364. doi:10.1016/j.phymed.2025.156364 PubMed PMID: 39862791.
69. Chen C, Li J, Wen B, Dou W, Zhu H. Integrative meta-analysis and experimental validation reveal the oncogenic role of SACS and its therapeutic targeting potential in colorectal cancer. *Sci Rep*. 2025 Nov 11;15(1):39522. doi:10.1038/s41598-025-23339-7
70. Wu C, Pan J, Gong B, Liao Y, Zou Y, Wang Y, et al. Immunochemical and LC–MS/MS Detection of Protein Adduction Resulting from Metabolic Activation of Columbin In Vitro and

In Vivo. Chem Res Toxicol. 2023 Apr 17;36(4):691–702. doi:10.1021/acs.chemrestox.3c00006

71. Moody JO, Robert VA, Connolly JD, Houghton PJ. Anti-inflammatory activities of the methanol extracts and an isolated furanoditerpene constituent of *Sphenocentrum jollyanum* Pierre (Menispermaceae). J Ethnopharmacol. 2006 Mar 8;104(1–2):87–91. doi:10.1016/j.jep.2005.08.051 PubMed PMID: 16236477.
72. A Y, Rs C, Am S, Te P. Semisynthesis and Kappa-Opioid Receptor Activity of Derivatives of Columbin, a Furanolactone Diterpene. J Nat Prod. 2017 Jul 28;80(7). doi:10.1021/acs.jnatprod.7b00327 PubMed PMID: 28718638.
73. Chaudhary A, Das R, Mehta K, Mehta DK. Indian herb *Tinospora cordifolia* and *Tinospora* species: Phytochemical and therapeutic application. Heliyon. 2024 May 30;10(10):e31229. doi:10.1016/j.heliyon.2024.e31229 PubMed PMID: 38813196; PubMed Central PMCID: PMC11133831.
74. Sharma P, Dwivedee BP, Bisht D, Dash AK, Kumar D. The chemical constituents and diverse pharmacological importance of *Tinospora cordifolia*. Heliyon. 2019 Sep 12;5(9):e02437. doi:10.1016/j.heliyon.2019.e02437 PubMed PMID: 31701036; PubMed Central PMCID: PMC6827274.
75. Mathe A, Mulpuru V, Katari SK, Karlapudi AP, T C V. Virtual screening and invitro evaluation of cyclooxygenase inhibitors from *Tinospora cordifolia* using the machine learning tool. J Biomol Struct Dyn. 2024;42(23):13275–89. doi:10.1080/07391102.2023.2275175 PubMed PMID: 37904339.
76. Arunachalam K, Yang X, San TT. *Tinospora cordifolia* (Willd.) Miers: Protection mechanisms and strategies against oxidative stress-related diseases. J Ethnopharmacol. 2022 Jan 30;283:114540. doi:10.1016/j.jep.2021.114540 PubMed PMID: 34509604.
77. George G, Shyni GL, Mohan S, Abraham B, Nisha P, Ranjith S, et al. In vitro and in vivo anti-inflammatory and anti-arthritic effect of *Tinospora cordifolia* via modulation of JAK/STAT pathway. Inflammopharmacology. 2023 Apr;31(2):1009–25. doi:10.1007/s10787-023-01155-7 PubMed PMID: 36840884.
78. Hopkins AL. Network pharmacology: the next paradigm in drug discovery. Nat Chem Biol. 2008 Nov;4(11):682–90. doi:10.1038/nchembio.118 PubMed PMID: 18936753.

79. Zhang R, Zhu X, Bai H, Ning K. Network Pharmacology Databases for Traditional Chinese Medicine: Review and Assessment. *Front Pharmacol.* 2019 Feb 21;10. doi:10.3389/fphar.2019.00123
80. Nogales C, Mamdouh ZM, List M, Kiel C, Casas AI, Schmidt HHHW. Network pharmacology: curing causal mechanisms instead of treating symptoms. *Trends Pharmacol Sci.* 2022 Feb;43(2):136–50. doi:10.1016/j.tips.2021.11.004 PubMed PMID: 34895945.
81. Zhang G biao, Li Q ya, Chen Q long, Su S bing. Network Pharmacology: A New Approach for Chinese Herbal Medicine Research. *Evid-Based Complement Altern Med ECAM.* 2013;2013:621423. doi:10.1155/2013/621423 PubMed PMID: 23762149; PubMed Central PMCID: PMC3671675.
82. Li L, Yang L, Yang L, He C, He Y, Chen L, et al. Network pharmacology: a bright guiding light on the way to explore the personalized precise medication of traditional Chinese medicine. *Chin Med.* 2023 Nov 8;18(1):146. doi:10.1186/s13020-023-00853-2 PubMed PMID: 37941061; PubMed Central PMCID: PMC10631104.
83. Vos T, Lim SS, Abbafati C, Abbas KM, Abbasi M, Abbasifard M, et al. Global burden of 369 diseases and injuries in 204 countries and territories, 1990–2019: a systematic analysis for the Global Burden of Disease Study 2019. *The Lancet.* 2020 Oct 17;396(10258):1204–22. doi:10.1016/S0140-6736(20)30925-9 PubMed PMID: 33069326.
84. Joshi CP, Baldi A, Kumar N, Pradhan J. Harnessing network pharmacology in drug discovery: an integrated approach. *Naunyn Schmiedebergs Arch Pharmacol.* 2025 May;398(5):4689–703. doi:10.1007/s00210-024-03625-3 PubMed PMID: 39621088.
85. Zimmermann GR, Lehár J, Keith CT. Multi-target therapeutics: when the whole is greater than the sum of the parts. *Drug Discov Today.* 2007 Jan;12(1–2):34–42. doi:10.1016/j.drudis.2006.11.008 PubMed PMID: 17198971.
86. Kabir A, Muth A. Polypharmacology: The science of multi-targeting molecules. *Pharmacol Res.* 2022 Feb;176:106055. doi:10.1016/j.phrs.2021.106055 PubMed PMID: 34990865.
87. Ryszkiewicz P, Malinowska B, Schlicker E. Polypharmacology: promises and new drugs in 2022. *Pharmacol Rep PR.* 2023 Aug;75(4):755–70. doi:10.1007/s43440-023-00501-4 PubMed PMID: 37278927; PubMed Central PMCID: PMC10243259.
88. Zhao L, Zhang H, Li N, Chen J, Xu H, Wang Y, et al. Network pharmacology, a promising approach to reveal the pharmacology mechanism of Chinese medicine formula. *J*

- Ethnopharmacol. 2023 Jun 12;309:116306. doi:10.1016/j.jep.2023.116306 PubMed PMID: 36858276.
89. Lee WY, Lee CY, Kim YS, Kim CE. The Methodological Trends of Traditional Herbal Medicine Employing Network Pharmacology. *Biomolecules*. 2019 Aug 13;9(8):362. doi:10.3390/biom9080362 PubMed PMID: 31412658; PubMed Central PMCID: PMC6723118.
 90. Zhai Y, Liu L, Zhang F, Chen X, Wang H, Zhou J, et al. Network pharmacology: a crucial approach in traditional Chinese medicine research. *Chin Med*. 2025 Jan 12;20(1):8. doi:10.1186/s13020-024-01056-z PubMed PMID: 39800680; PubMed Central PMCID: PMC11725223.
 91. Nureye D. Reverse Pharmacology and Network Pharmacology: Principles and Applications in Herbal Medicine. *J Tradit Med Appl*. 2024 Nov 21;3(2):1–24.
 92. Liu Y, Li X, Chen C, Ding N, Ma S, Yang M. Exploration of compatibility rules and discovery of active ingredients in TCM formulas by network pharmacology. *Chin Herb Med*. 2024 Apr 2;16(4):572–88. doi:10.1016/j.chmed.2023.09.008 PubMed PMID: 39606260; PubMed Central PMCID: PMC11589340.
 93. Kibble M, Saarinen N, Tang J, Wennerberg K, Mäkelä S, Aittokallio T. Network pharmacology applications to map the unexplored target space and therapeutic potential of natural products. *Nat Prod Rep*. 2015 Aug;32(8):1249–66. doi:10.1039/c5np00005j PubMed PMID: 26030402.
 94. Guedes IA, de Magalhães CS, Dardenne LE. Receptor-ligand molecular docking. *Biophys Rev*. 2014 Mar;6(1):75–87. doi:10.1007/s12551-013-0130-2 PubMed PMID: 28509958; PubMed Central PMCID: PMC5425711.
 95. Du X, Li Y, Xia YL, Ai SM, Liang J, Sang P, et al. Insights into Protein-Ligand Interactions: Mechanisms, Models, and Methods. *Int J Mol Sci*. 2016 Jan 26;17(2):144. doi:10.3390/ijms17020144 PubMed PMID: 26821017; PubMed Central PMCID: PMC4783878.
 96. Pagadala NS, Syed K, Tuszynski J. Software for molecular docking: a review. *Biophys Rev*. 2017 Apr;9(2):91–102. doi:10.1007/s12551-016-0247-1 PubMed PMID: 28510083; PubMed Central PMCID: PMC5425816.

97. Li J, Fu A, Zhang L. An Overview of Scoring Functions Used for Protein-Ligand Interactions in Molecular Docking. *Interdiscip Sci Comput Life Sci.* 2019 Jun;11(2):320–8. doi:10.1007/s12539-019-00327-w PubMed PMID: 30877639.
98. Yang C, Chen EA, Zhang Y. Protein-Ligand Docking in the Machine-Learning Era. *Molecules.* 2022 Jul 18;27(14):4568. doi:10.3390/molecules27144568 PubMed PMID: 35889440; PubMed Central PMCID: PMC9323102.
99. Sousa SF, Fernandes PA, Ramos MJ. Protein-ligand docking: current status and future challenges. *Proteins.* 2006 Oct 1;65(1):15–26. doi:10.1002/prot.21082 PubMed PMID: 16862531.
100. Mf A, Kl L, Sn B, F K, S S, Vj H, et al. PLIP 2021: expanding the scope of the protein-ligand interaction profiler to DNA and RNA. *Nucleic Acids Res.* 2021 Jul 2;49(W1). doi:10.1093/nar/gkab294 PubMed PMID: 33950214.
101. Schöning-Stierand K, Diedrich K, Fährrolfes R, Flachsenberg F, Meyder A, Nittinger E, et al. ProteinsPlus: interactive analysis of protein-ligand binding interfaces. *Nucleic Acids Res.* 2020 Jul 2;48(W1):W48–53. doi:10.1093/nar/gkaa235 PubMed PMID: 32297936; PubMed Central PMCID: PMC7319454.
102. Wang Z, Sun H, Yao X, Li D, Xu L, Li Y, et al. Comprehensive evaluation of ten docking programs on a diverse set of protein–ligand complexes: the prediction accuracy of sampling power and scoring power. *Phys Chem Chem Phys.* 2016 May 4;18(18):12964–75. doi:10.1039/C6CP01555G
103. Burris DM, Gillespie SW, Campbell EJ, Ice SN, Yadav V, Picking WD, et al. Applications of Surface Plasmon Resonance (SPR) to the Study of Diverse Protein-Ligand Interactions. *Curr Protoc.* 2024 Jun;4(6):e1030. doi:10.1002/cpz1.1030 PubMed PMID: 38923763.
104. Grijaldo SJB, Alvarez MRS, Heralde FMI, Nacario RC, Lebrilla CB, Rabajante JF, et al. Integrating Computational Methods in Network Pharmacology and In Silico Screening to Uncover Multi-targeting Phytochemicals against Aberrant Protein Glycosylation in Lung Cancer. *ACS Omega.* 2023 Jun 13;8(23):20303–12. doi:10.1021/acsomega.2c07542
105. Azeem K, Abdulhameed HT, Hussain A, Amir S, Parveen M, Patel R, et al. A Comprehensive Multispectroscopic and Computational Analysis of the Interaction between Plant-Based Antiplasmodial Compounds and Bovine Serum Albumin. *ACS Omega.* 2024 Jan 28;9(5):5576. doi:10.1021/acsomega.3c07630 PubMed PMID: 38343956.

106. Sy H, X Z. Advances and challenges in protein-ligand docking. *Int J Mol Sci.* 2010 Aug 18;11(8). doi:10.3390/ijms11083016 PubMed PMID: 21152288.
107. Nezamabadi Farahani L, Kazemnejad A, Afrasiabi M, Tapak L. In Silico Transcriptomic Analysis for Identification of Potential Diagnostic and Prognostic Biomarkers and Therapeutic Targets in Cervical Cancer using a Hybrid Genetic Algorithm–Support Vector Machine Approach. *Arch Iran Med.* 2025 Dec;28(12):677–86. doi:10.34172/aim.34814 PubMed PMID: 41852002; PubMed Central PMCID: PMC13000335.
108. Altaf R, Ilyas U, Ma A, Shi M. Identification and validation of differentially expressed genes for targeted therapy in NSCLC using integrated bioinformatics analysis. *Front Oncol.* 2023 May 31;13. doi:10.3389/fonc.2023.1206768
109. Oli M. MK, Syed Masood JAI. Identification and validation of tumor microenvironment-related therapeutic targets in gastric cancer using integrated multi-omics and molecular docking approaches. *Front Bioinforma.* 2025 Dec 10;5. doi:10.3389/fbinf.2025.1654326
110. Ziani PR, de Bastiani MA, Scotton E, da Rosa PH, Schons T, Mezzomo G, et al. Drug repurposing and personalized treatment strategies for bipolar disorder using transcriptomics: an exploratory study. *Braz J Psychiatry.* 2024 Aug 19;46:e20233441. doi:10.47626/1516-4446-2023-3441 PubMed PMID: 38446713; PubMed Central PMCID: PMC11488474.
111. Zhang HE, Xiao ML, Ji JJ, Cheng YR, Lu F. Deciphering Alzheimer’s disease transcriptomics: exploration and validation of core genes in tau and A β pathological models toward novel therapeutic targets. *Front Aging Neurosci.* 2025 Oct 10;17. doi:10.3389/fnagi.2025.1621153
112. Melouane A, Ghanemi A, Aubé S, Yoshioka M, St-Amand J. Differential gene expression analysis in ageing muscle and drug discovery perspectives. *Ageing Res Rev.* 2018 Jan;41:53–63. doi:10.1016/j.arr.2017.10.006 PubMed PMID: 29102726.
113. Rentzsch P, Kollotzek A, Mohammadi P, Lappalainen T. Recalibrating differential gene expression by genetic dosage variance prioritizes functionally relevant genes. *BioRxiv Prepr Serv Biol.* 2024 Apr 10;2024.04.10.588830. doi:10.1101/2024.04.10.588830 PubMed PMID: 38645217; PubMed Central PMCID: PMC11030425.
114. Rosati D, Palmieri M, Brunelli G, Morrione A, Iannelli F, Frullanti E, et al. Differential gene expression analysis pipelines and bioinformatic tools for the identification of specific biomarkers: A review. *Comput Struct Biotechnol J.* 2024 Dec;23:1154–68.

doi:10.1016/j.csbj.2024.02.018 PubMed PMID: 38510977; PubMed Central PMCID: PMC10951429.

115. Wang Y, Yang HY, Fan ZA, Zhang JC. Machine learning-driven exploration of therapeutic targets for atrial fibrillation-joint analysis of single-cell and bulk transcriptomes and experimental validation. *Front Cardiovasc Med.* 2025 Nov 3;12. doi:10.3389/fcvm.2025.1652467
116. Liao Z, Jia P, Li Y, Zheng Z, Zhang J. Exploring potential therapeutic targets for small cell lung cancer based on transcriptomics combined with Mendelian randomization analysis. *Front Immunol.* 2025 Jan 13;15. doi:10.3389/fimmu.2024.1464259
117. Liu C, Li Z, Xi H. Bioinformatics analysis and in vivo validation of ferroptosis-related genes in ischemic stroke. *Front Pharmacol.* 2022 Nov 24;13. doi:10.3389/fphar.2022.940260
118. Larriba E, de Juan Romero C, García-Martínez A, Quintanar T, Rodríguez-Lescure Á, Soto JL, et al. Identification of new targets for glioblastoma therapy based on a DNA expression microarray. *Comput Biol Med.* 2024 Sep;179:108833. doi:10.1016/j.combiomed.2024.108833 PubMed PMID: 38981212.
119. Feng Z wei, Yang M kun, Jia X dong, Yuan F, Guo M gang, Chen F, et al. Unveiling potential diagnostic biomarkers for rheumatoid arthritis through integrated gene expression analysis. *Front Immunol.* 2026 Feb 24;17. doi:10.3389/fimmu.2026.1645257
120. Cui F, Liu R, Wang L, He J, Xu Y. Identification and validation of differentially expressed genes in allergic asthma pathogenesis using whole-transcriptome sequencing. *Front Med.* 2025 Apr 16;12. doi:10.3389/fmed.2025.1545095
121. Isiyel M, Ceylan H, Demir Y. Bioinformatics-Based Discovery of Therapeutic Targets in Cadmium-Induced Lung Adenocarcinoma: The Role of Oxyresveratrol. *Biol Trace Elem Res.* 2026;204(2):1068–83. doi:10.1007/s12011-025-04730-x PubMed PMID: 40610695; PubMed Central PMCID: PMC12847123.
122. Bartha Á, Györffy B. TNMplot.com: A Web Tool for the Comparison of Gene Expression in Normal, Tumor and Metastatic Tissues. *Int J Mol Sci.* 2021 Mar 5;22(5):2622. doi:10.3390/ijms22052622 PubMed PMID: 33807717; PubMed Central PMCID: PMC7961455.

123. Liao Y, Wang X, Ran G, Zhang S, Wu C, Tan R, et al. DNA damage and up-regulation of PARP-1 induced by columbin in vitro and in vivo. *Toxicol Lett.* 2023 Apr 15;379:20–34. doi:10.1016/j.toxlet.2023.03.003 PubMed PMID: 36905973.
124. Furman D, Campisi J, Verdin E, Carrera-Bastos P, Targ S, Franceschi C, et al. Chronic inflammation in the etiology of disease across the life span. *Nat Med.* 2019 Dec;25(12):1822–32. doi:10.1038/s41591-019-0675-0
125. Medzhitov R. Origin and physiological roles of inflammation. *Nature.* 2008 Jul 24;454(7203):428–35. doi:10.1038/nature07201 PubMed PMID: 18650913.
126. T L. The nuclear factor NF-kappaB pathway in inflammation. *Cold Spring Harb Perspect Biol.* 2009 Dec;1(6). doi:10.1101/cshperspect.a001651 PubMed PMID: 20457564.
127. Saha S, Ghosh S. *Tinospora cordifolia*: One plant, many roles. *Anc Sci Life.* 2012 Apr;31(4):151–9. doi:10.4103/0257-7941.107344 PubMed PMID: 23661861; PubMed Central PMCID: PMC3644751.
128. Zhang L, Lu S. Overview of Medicinally Important Diterpenoids Derived from Plastids. *Mini Rev Med Chem.* 2017;17(12):988–1001. doi:10.2174/1389557516666160614005244 PubMed PMID: 27297674.
129. Bao H, Zhang Q, Ye Y, Lin L. Naturally occurring furanoditerpenoids: distribution, chemistry and their pharmacological activities. *Phytochem Rev.* 2017 Apr 1;16(2):235–70. doi:10.1007/s11101-016-9472-2
130. Hopkins AL. Network pharmacology: the next paradigm in drug discovery. *Nat Chem Biol.* 2008 Nov;4(11):682–90. doi:10.1038/nchembio.118 PubMed PMID: 18936753.
131. Li S, Zhang B. Traditional Chinese medicine network pharmacology: theory, methodology and application. *Chin J Nat Med.* 2013 Mar;11(2):110–20. doi:10.1016/S1875-5364(13)60037-0 PubMed PMID: 23787177.
132. Barrett T, Wilhite SE, Ledoux P, Evangelista C, Kim IF, Tomashevsky M, et al. NCBI GEO: archive for functional genomics data sets--update. *Nucleic Acids Res.* 2013 Jan;41(Database issue):D991-995. doi:10.1093/nar/gks1193 PubMed PMID: 23193258; PubMed Central PMCID: PMC3531084.
133. Daina A, Michielin O, Zoete V. SwissTargetPrediction: updated data and new features for efficient prediction of protein targets of small molecules. *Nucleic Acids Res.* 2019 Jul 2;47(W1):W357–64. doi:10.1093/nar/gkz382

134. Yao ZJ, Dong J, Che YJ, Zhu MF, Wen M, Wang NN, et al. TargetNet: a web service for predicting potential drug–target interaction profiling via multi-target SAR models. *J Comput Aided Mol Des*. 2016 May 1;30(5):413–24. doi:10.1007/s10822-016-9915-2
135. Liu X, Ouyang S, Yu B, Liu Y, Huang K, Gong J, et al. PharmMapper server: a web server for potential drug target identification using pharmacophore mapping approach. *Nucleic Acids Res*. 2010 Jul;38(Web Server issue):W609-614. doi:10.1093/nar/gkq300 PubMed PMID: 20430828; PubMed Central PMCID: PMC2896160.
136. Gallo K, Goede A, Preissner R, Gohlke BO. SuperPred 3.0: drug classification and target prediction-a machine learning approach. *Nucleic Acids Res*. 2022 Jul 5;50(W1):W726–31. doi:10.1093/nar/gkac297 PubMed PMID: 35524552; PubMed Central PMCID: PMC9252837.
137. Stelzer G, Rosen N, Plaschkes I, Zimmerman S, Twik M, Fishilevich S, et al. The GeneCards Suite: From Gene Data Mining to Disease Genome Sequence Analyses. *Curr Protoc Bioinforma*. 2016 Jun 20;54:1.30.1-1.30.33. doi:10.1002/cpbi.5 PubMed PMID: 27322403.
138. Piñero J, Ramírez-Anguita JM, Saüch-Pitarch J, Ronzano F, Centeno E, Sanz F, et al. The DisGeNET knowledge platform for disease genomics: 2019 update. *Nucleic Acids Res*. 2020 Jan 8;48(D1):D845–55. doi:10.1093/nar/gkz1021 PubMed PMID: 31680165; PubMed Central PMCID: PMC7145631.
139. Q H, C L, X W, K R, M Z, L D, et al. Exploring the mechanism of curcumin in the treatment of colon cancer based on network pharmacology and molecular docking. *Front Pharmacol*. 2023 Feb 15;14. doi:10.3389/fphar.2023.1102581 PubMed PMID: 36874006.
140. Woetzel D, Huber R, Kupfer P, Pohlars D, Pfaff M, Driesch D, et al. Identification of rheumatoid arthritis and osteoarthritis patients by transcriptome-based rule set generation. *Arthritis Res Ther*. 2014 Apr 1;16(2):R84. doi:10.1186/ar4526 PubMed PMID: 24690414; PubMed Central PMCID: PMC4060460.
141. Oliveros JC. Venny. An interactive tool for comparing lists with Venn's diagrams. [Internet]. 2007 2015. Available from: <https://bioinfogp.cnb.csic.es/tools/venny/index.html>
142. Szklarczyk D, Kirsch R, Koutrouli M, Nastou K, Mehryary F, Hachilif R, et al. The STRING database in 2023: protein-protein association networks and functional enrichment analyses for any sequenced genome of interest. *Nucleic Acids Res*. 2023 Jan 6;51(D1):D638–

46. doi:10.1093/nar/gkac1000 PubMed PMID: 36370105; PubMed Central PMCID: PMC9825434.
143. Franz M, Lopes CT, Huck G, Dong Y, Sumer O, Bader GD. Cytoscape.js: a graph theory library for visualisation and analysis. *Bioinformatics*. 2016 Jan 15;32(2):309–11. doi:10.1093/bioinformatics/btv557 PubMed PMID: 26415722; PubMed Central PMCID: PMC4708103.
144. Sherman BT, Hao M, Qiu J, Jiao X, Baseler MW, Lane HC, et al. DAVID: a web server for functional enrichment analysis and functional annotation of gene lists (2021 update). *Nucleic Acids Res*. 2022 Jul 5;50(W1):W216–21. doi:10.1093/nar/gkac194 PubMed PMID: 35325185; PubMed Central PMCID: PMC9252805.
145. O’Boyle NM, Banck M, James CA, Morley C, Vandermeersch T, Hutchison GR. Open Babel: An open chemical toolbox. *J Cheminformatics*. 2011 Oct 7;3:33. doi:10.1186/1758-2946-3-33 PubMed PMID: 21982300; PubMed Central PMCID: PMC3198950.
146. Zhang M, Jang H, Nussinov R. Structural Features that Distinguish Inactive and Active PI3K Lipid Kinases. *J Mol Biol*. 2020 Nov 6;432(22):5849–59. doi:10.1016/j.jmb.2020.09.002 PubMed PMID: 32918948; PubMed Central PMCID: PMC8916166.
147. Eberhardt J, Santos-Martins D, Tillack AF, Forli S. AutoDock Vina 1.2.0: New Docking Methods, Expanded Force Field, and Python Bindings. *J Chem Inf Model*. 2021 Aug 23;61(8):3891–8. doi:10.1021/acs.jcim.1c00203
148. PyMOL | pymol.org [Internet]. [cited 2026 Apr 16]. Available from: <https://www.pymol.org/>
149. Discovery Studio. San Diego: Dassault Systèmes: BIOVIA, Dassault Systèmes; 2025.
150. Bugnon M, Röhrig UF, Goullieux M, Perez MAS, Daina A, Michielin O, et al. SwissDock 2024: major enhancements for small-molecule docking with Attracting Cavities and AutoDock Vina. *Nucleic Acids Res*. 2024 Jul 5;52(W1):W324–32. doi:10.1093/nar/gkac300 PubMed PMID: 38686803; PubMed Central PMCID: PMC11223881.
151. Newman AM, Liu CL, Green MR, Gentles AJ, Feng W, Xu Y, et al. Robust enumeration of cell subsets from tissue expression profiles. *Nat Methods*. 2015 May;12(5):453–7. doi:10.1038/nmeth.3337 PubMed PMID: 25822800; PubMed Central PMCID: PMC4739640.

152. Woetzel D, Huber R, Kupfer P, Pohlers D, Pfaff M, Driesch D, et al. Identification of rheumatoid arthritis and osteoarthritis patients by transcriptome-based rule set generation. *Arthritis Res Ther*. 2014 Apr 1;16(2):R84. doi:10.1186/ar4526 PubMed PMID: 24690414; PubMed Central PMCID: PMC4060460.
153. Ritchie ME, Phipson B, Wu D, Hu Y, Law CW, Shi W, et al. limma powers differential expression analyses for RNA-sequencing and microarray studies. *Nucleic Acids Res*. 2015 Apr 20;43(7):e47. doi:10.1093/nar/gkv007
154. Robin X, Turck N, Hainard A, Tiberti N, Lisacek F, Sanchez JC, et al. pROC: an open-source package for R and S+ to analyze and compare ROC curves. *BMC Bioinformatics*. 2011 Mar 17;12(1):77. doi:10.1186/1471-2105-12-77
155. Daina A, Michielin O, Zoete V. SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. *Sci Rep*. 2017 Mar 3;7(1):42717. doi:10.1038/srep42717
156. Pires DEV, Blundell TL, Ascher DB. pkCSM: Predicting Small-Molecule Pharmacokinetic and Toxicity Properties Using Graph-Based Signatures. *J Med Chem*. 2015 May 14;58(9):4066–72. doi:10.1021/acs.jmedchem.5b00104
157. Banerjee P, Kemmler E, Dunkel M, Preissner R. ProTox 3.0: a webserver for the prediction of toxicity of chemicals. *Nucleic Acids Res*. 2024 Jul 5;52(W1):W513–20. doi:10.1093/nar/gkae303



DELHI TECHNOLOGICAL UNIVERSITY

(Formerly Delhi College of Engineering)

Shahbad Daulatpur, Main Bawana Road, Delhi-42

PLAGIARISM VERIFICATION

Title of the thesis: Integrated Network Pharmacology, Transcriptomic Validation,
And Molecular Docking Analysis Of Anti-Inflammatory Mechanisms Of
Columbin

Total Pages: 64

Name of the Student: Ananya Singh

Supervisor: Prof. Yasha Hasija

Department of Biotechnology, Delhi Technological University, Delhi – 110042

This is to report that the above thesis was scanned for similarity detection. Process and outcome is given below:

Software used: Turnitin, **Similarity Index:** 7%, **Total Word Count:** 18373

Date: 28/06/2026

Candidate's Signature

Signature of Supervisor

ORIGINALITY REPORT

7 %

SIMILARITY INDEX

5 %

INTERNET SOURCES

5 %

PUBLICATIONS

1 %

STUDENT PAPERS

PRIMARY SOURCES

1	www.frontiersin.org Internet Source	1 %
2	public-pages-files-2025.frontiersin.org Internet Source	1 %
3	www.mdpi.com Internet Source	<1 %
4	pmc.ncbi.nlm.nih.gov Internet Source	<1 %
5	www.science.gov Internet Source	<1 %
6	www.benthamdirect.com Internet Source	<1 %
7	academic.oup.com Internet Source	<1 %
8	Debasis Bagchi, Kiran Bhupathiraju, Samudra Prosad Banik. "Health Benefits of (Shatavari) - A Comprehensive Guide to the Medicinal and Nutritional Potential of Shatavari", CRC Press, 2026	<1 %

*% detected as AI

AI detection includes the possibility of false positives. Although some text in this submission is likely AI generated, scores below the 20% threshold are not surfaced because they have a higher likelihood of false positives.

Caution: Review required.

It is essential to understand the limitations of AI detection before making decisions about a student's work. We encourage you to learn more about Turnitin's AI detection capabilities before using the tool.

Disclaimer

Our AI writing assessment is designed to help educators identify text that might be prepared by a generative AI tool. Our AI writing assessment may not always be accurate (i.e., our AI models may produce either false positive results or false negative results), so it should not be used as the sole basis for adverse actions against a student. It takes further scrutiny and human judgment in conjunction with an organization's application of its specific academic policies to determine whether any academic misconduct has occurred.