SYNERGIZING IN-SILICO APPROACHES OF NATURAL PRODUCT-BASED DRUG DISCOVERY AND DRUG REPURPOSING TO TARGET STATS IN GLIOBLASTOMA THERAPEUTICS

Thesis Submitted
in Partial Fulfillment of the Requirements for the
Degree of

MASTER OF SCIENCE
in
BIOTECHNOLOGY
by

NANCY
(2K22/MSCBIO/32)

Under the Supervision of
Prof. Pravir Kumar
Professor and Dean IA
Delhi Technological University

To the
Department of Biotechnology
DELHI TECHNOLOGICAL UNIVERSITY
(Formerly Delhi College of Engineering)
Shahbad Daulatpur, Main Bawana Road, Delhi-110042, India

June, 2024

SYNERGIZING IN-SILICO APPROACHES OF NATURAL PRODUCT-BASED DRUG DISCOVERY AND DRUG REPURPOSING TO TARGET STATS IN GLIOBLASTOMA THERAPEUTICS

Thesis Submitted
in Partial Fulfillment of the Requirements for the
Degree of

MASTER OF SCIENCE

in

BIOTECHNOLOGY

by

NANCY
(2K22/MSCBIO/32)

Under the Supervision of
Prof. Pravir Kumar
Professor and Dean IA
Delhi Technological University

To the
Department of Biotechnology
DELHI TECHNOLOGICAL UNIVERSITY
(Formerly Delhi College of Engineering)
Shahbad Daulatpur, Main Bawana Road, Delhi-110042, India

June, 2024

ACKNOWLEDGEMENT

I am pleased to present this dissertation project as part of my curriculum and deeply grateful for the divine guidance that endowed me with the wisdom, resilience, and endurance needed to complete this journey. While my dedication played a vital role, the support and guidance of many individuals were crucial to the project's success. I extend my heartfelt appreciation to everyone who contributed to the successful completion of this endeavor.

Firstly, I sincerely appreciate my mentor, Prof. Pravir Kumar from the Department of Biotechnology at Delhi Technological University, for entrusting me with this opportunity. His exceptional mentorship, unwavering support, and invaluable guidance have been crucial throughout all stages of this project. His expertise, patience, and commitment to excellence have significantly shaped the direction and quality of this work, and his insightful feedback has been instrumental in the successful completion of this work.

I am equally appreciative and extend my sincere gratitude to Ms. Mehar Sahu and Mrs. Neetu. Their exceptional guidance, insightful feedback, unwavering belief in my abilities, and familial support served as a consistent source of motivation throughout my project. Their profound commitment to nurturing intellectual curiosity and fostering academic growth has greatly enriched my journey as a researcher.

In conclusion, I wish to extend my profound gratitude to my family and friends, whose unwavering support has served as a steadfast pillar of strength throughout this remarkable journey. Their unwavering belief in my abilities, patience during moments of adversity, and continuous encouragement have been an infinite source of motivation and inspiration. I am also deeply indebted to my colleagues and friends for their invaluable assistance, generously sharing their expertise, resources, and time. Their collaborative spirit, stimulating intellectual discussions, and unwavering moral support have not only made this journey immensely rewarding but have also played an indispensable role in refining my ideas and broadening the horizons of this work. Lastly, I extend heartfelt appreciation to all individuals whose contributions, though not explicitly mentioned, have left an indelible mark on this thesis. Your invaluable support and impact have been instrumental, and for that, I am profoundly grateful.

NANCY

2K22/MSCBIO/32

DELHI TECHNOLOGICAL UNIVERSITY
(Formerly Delhi College of Engineering)
Shahbad Daulatpur, Main Bawana Road, Delhi-110042

CANDIDATE'S DECLARATION

I, Nancy hereby certify that the work which is being presented in the thesis entitled **"Synergizing In-Silico Approaches of Natural Product-Based Drug Discovery and Drug Repurposing to Target STATs in Glioblastoma Therapeutics"** in partial fulfilment of the requirement for the award of the Degree of Master of Science, submitted in the Department of Biotechnology, Delhi Technological University is an authentic record of my own work carried out during the period from January 2024 to May 2024 under the supervision of Prof. Pravir Kumar.

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

Nancy

Candidate's Signature

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

05/06/2024

Signature of Supervisor

DELHI TECHNOLOGICAL UNIVERSITY

(Formerly Delhi College of Engineering)
Shahbad Daulatpur, Main Bawana Road, Delhi-110042

CERTIFICATE BY THE SUPERVISOR

Certified that **Nancy** (2K22/MSCBIO/32) has carried out their search work presented in this thesis entitled "**Synergizing In-Silico Approaches of Natural Product-Based Drug Discovery and Drug Repurposing to Target STATs in Glioblastoma Therapeutics**" for the award of **Master of Science** from Department of Biotechnology, 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 reward of any other degree to the candidate or to anybody else from this or any other University/Institution.

05/06/2024

Prof. Pravir Kumar
Supervisor, Dean IA
Department of Biotechnology
Delhi Technological University

05.06.24

Prof. Yasha Hasija
Head of Department
Department of Biotechnology
Delhi Technological University

Date:

Synergizing In-Silico Approaches of Natural Product-Based Drug Discovery and Drug Repurposing to Target STATs in Glioblastoma Therapeutics

NANCY

ABSTRACT

Background

Glioblastoma, or Glioblastoma multiforme (GBM), is a primary central nervous system glioma that remains incurable due to the lack of effective treatments. This approach, encompassing both drug discovery and drug repurposing, presents a promising strategy against GBM. The JAK/STAT signaling cascade holds a pivotal role in various tumor-promoting functions and is implicated in the pathogenesis and progression of GBM. This study aims to inhibit this pathway by targeting STAT3 and STAT1 using an in-silico approach to identify potential inhibitors. The research focuses discovering the phytocompounds derived from various Indian medicinal plants known for their therapeutic applications and re-purposing FDA approved drugs directly derived from Drug Bank database. Phytochemicals from these plants were manually collected and curated based on their blood-brain barrier permeability using Swiss ADME. Molecular docking was conducted with PyRx, and docking scores were validated through CB-Dock2. Binding interactions were visualized with PyMOL and Discovery Studio. Additionally, ADMET analysis was performed using Swiss ADME and the PkCSM tool, while carcinogenicity and toxicity to cancer cell line was assessed with CarcinoPred-EL and CLC-Pred 2.0.

Results

Five phytocompounds, withametelin, isowithametelin, anolobine, withasomidienone and xylopine along with three FDA approved tirilazad, telmisartan and mizolastine were identified with some inhibitors showing promising pharmacological properties such as blood-brain barrier permeability, against STAT3. Most of these compounds also demonstrated strong binding affinity with STAT1 as well.

Conclusion

Some of the compounds derived from selected medicinal plants and few FDA approved drugs have the potential for being the potential drug candidates in the treatment of GBM. Their favorable docking scores indicate strong binding with targets and effective drug-like properties, qualify them as potential inhibitors of both STAT3 and STAT1.

LIST OF PUBLICATIONS

Title of Paper - "Navigating Glioblastoma Therapy: A Computational and Drug Repurposing Approach Targeting STAT3"

Author Names- Nancy and Prof. Pravir Kumar

Name of the Conference- 5th International Conference for Emerging Technology (INCET) 2024, Belgaum, India.

Date of Conference- 24th May – 26th May 2024

Indexing- IEEE

Status of Paper- Accepted

Date of Acceptance- 8th March 2024

Date of Camera-Ready Submission and Registration- 28th March 2024 and 15th March 2024

TABLE OF CONTENTS

Title	Page No.
Abstract	**iv**
List of Publication	**v**
Table of Contents	**vi-vii**
List of Tables	**viii**
List of Figures	**ix**
List of abbreviations	**x**
CHAPTER 1: INTRODUCTION AND LITERATURE REVIEW	**1-8**
1.1. Overview	1-2
1.2. Glioblastoma	2
1.2.1 Basic Outline	2
1.2.2 Clinical Presentation and Distribution of Glioblastoma	2-3
1.2.3 Histopathology & Pathphysiology	3
1.2.4 Treatment Strategies	3-4
1.3. JAK-STAT Signalling Pathway	4
1.3.1 General Review	4
1.3.2 Components	4-5
1.3.3 Physiology	5
1.3.4 STAT3 and GBM	6-7
1.3.5 STAT1 and GBM	7-8
CHAPTER 2: METHODOLOGY	**9-15**
2.1. Sources	9
2.2. Workflow	9
2.3. Molecular Docking	9
2.3.1 IMPPAT 2.0	9
2.3.2 Data Extraction	9-10
2.3.3 Ligand Preparation	13
2.3.4 Protein Preparation	13
2.3.5 PyRx	13
2.3.6 Docking	13-14
2.4. Docking Analysis	14
2.5. Docking Result Validation	14
2.6. Protein-Ligand Interaction Visualization	14
2.7. ADME Analysis	14
2.8. Toxicity and Carcinogenicity Analysis	14
2.9. In-silico based Cancer Cell Cytotoxicity Analysis	14-15
2.10. STAT1 Docking with Lead Compounds	15
CHAPTER 3: RESULT AND DISCUSSION	**16-28**
3.1. Result	16

3.1.1 Docking Result	16-17
3.1.2 Validation Result	17
3.1.3 Protein -Ligand Interaction Result	18
3.1.4 ADME Analysis Result	22
3.1.5 Toxicity and Carcinogenicity Analysis Result	25
3.1.6 Cancer Cell Line Toxicity Analysis Result	25
3.1.7 Docking Results with STAT1	27
3.2. Discussion	27-29
CHAPTER 4: CONCLUSION, FUTURE PROSPECTS AND SOCIAL IMPACT	**30-31**
REFRENCES	32-42
List of Publication and their proofs	43-51
Plagiarism Report	52-53
Curriculum Vitae	54-55

LIST OF TABLES

S. No.	Name of Table	Page No.
2.1	List of phytocompounds derived from IMPPAT 2.0	10-13
3.1	Binding affinities of compounds within threshold range	16-17
3.2	Leading FDA drugs exhibiting best affinity	17
3.3	Comparison of phytocompound binding scores from PyRx and CB-Dock2	17
3.4	Comparison of FDA approved drugs binding scores from PyRx and CB-Dock2	17
3.5	Interacting residues of STAT3 with leading phytocompounds and reference compound	18
3.6	Interacting amino acids of STAT3 with leading FDA drugs and reference compound	20
3.7	Physiochemical properties exhibited by selected phytocompounds	22
3.8	Drug likeliness and pharmacokinetic properties of leading phytocompounds	22
3.9	Drug likeliness and pharmacokinetic properties of leading phytocompounds	22
3.10	Physiochemical properties exhibited by selected FDA drugs	24
3.11	Drug likeliness and pharmacokinetic properties of leading FDA approved drugs	24
3.12	Drug likeliness and pharmacokinetic properties of leading FDA approved drugs	24
3.13	Toxicity and carcinogenicity exhibited by phytocompounds	25
3.14	Toxicity and carcinogenicity exhibited by FDA drugs	25
3.15	Cancer cell line toxicity shown by selected phytocompounds	26
3.16	Cancer cell line toxicity shown by selected FDA approved drugs	26
3.17	Comparison of docking scores for STAT1 with selected phytocompounds	27
3.18	Comparison of docking scores for STAT1 with selected FDA drugs	27

LIST OF FIGURES

S.No.	Title of Figures	Page No.
1.1	Comprehensive representation of STAT3 protein domains and their functional roles.	5
1.2	JAK/STAT Signaling Pathway	6
1.3	Illustration of STAT3 pivotal role with diverse tumor hallmarks	7
2.1	Overview of performed methodology	15
3.1	Representation of 3D interactions between the selected reference compounds and phytocompounds with STAT3	18-20
3.2	Representation of 3D interactions between the selected reference compounds and FDA drugs with STAT3	20-21
3.3	Bioavailability radar diagrams and BOILED-EGG images of phytocompounds derived by ADME analysis	23-24
3.4	Bioavailability radar diagrams and BOILED-EGG images of mizolastine derived by ADME analysis	25

LIST OF ABBREVIATIONS

Abbreviation	Page No.
GBM	Glioblastoma Multiforme
JAK/STAT	Janus Kinase/Signal Transducer and Activator of Transcription
ADMET	Absorption, Distribution, Metabolism, Excretion and Toxicity
WHO	World Health Organization
FDA	Food and Drug Administration
IDH	Isocitrate Dehydrogenase
BBB	Blood-Brain Barrier
JNK	c-Jun-NH(2)-Terminal Kinase
CAR T-cell	Chimeric Antigen Receptor T cell
CCD	Coiled-Coil Domain
DBD	DNA Binding Domain
TAD	Transcription-Activation Domain
BMX	Bone Marrow Kinase X
IGFBP-3	Insulin-like Growth Factor Binding Protein 3
HIF-1	Hypoxia-Inducible Factor 1
IFN-γ	Interferon-gamma
VEGF	Vascular Endothelial Growth Factor
IMPPAT 2.0	Indian Medicinal Plants, Phytochemistry and Therapeutics 2.0

CHAPTER - 1

INTRODUCTION AND LITERATURE REVIEW

1.1. Overview

Brain tumors are extremely aggressive and deadly forms of cancer with incidence rate of 6-7 cases per 100,000 people annually. Gliomas, comprising approximately 50% of all brain neoplasms, are predominant and most common type of cancer afflicting the central nervous system [1], [2]. Gliomas are infiltrative tumors originating from glial cells, affecting the adjacent brain tissue. Glial tumor infiltration leads to frequent recurrence, rendering gliomas resistant to diverse therapeutic approaches. These tumors are classified into distinct sub-types and grades, determined by histopathological characteristics, along with molecular and genetic markers. The principal types of gliomas include oligodendrogliomas, oligoastrocytomas, and astrocytomas, with astrocytomas being the most prevalent. Astrocytomas, deriving from astrocytes, are further classified into four grades based on their proliferative capacity and the severity of clinical severity [3], [4], [5]. Gliomas range from the least malignant Grade I, slow-progressing Grade II, rapidly progressing Grade III, to the most malignant Grade IV, also called glioblatoma or glioblastoma multiforme (GBM) categorised by WHO [6], [7].

Glioblastoma, the most prevalent glioma, ranks among the most aggressive tumors. It is characterized by a high recurrence rate and dismal survival statistics, with a median overall survival and mean progression-free survival of around 14 months and 7 months following diagnosis [8], [9]. Although the incidence of glioblastoma is considerably lower compared to other cancer types, these tumors have garnered significant attention due to their poor prognosis and the limited success of current treatments. Even after decades of substantial research and investment, there remains an urgent need for more effective treatments and novel therapeutic strategies for treatment of glioblastoma [10], [11]. Numerous factors contribute to the significant resistance of glioblastoma to existing therapies, with one of the primary causes being the dysregulation of signaling pathways that directly or indirectly drive tumorigenesis. Notably, the JAK/STAT pathway has emerged as a crucial molecular nexus in GBM cells, with increasing evidence underscoring its pivotal role in driving both tumor progression and treatment resistance [12].

Phytochemicals, characterized as non-conventional nutrients found in plant-based foods, offer protective effects with minimal side effects, playing pivotal roles in disease prevention. A notable area of interest lies in exploring the relationship between phytochemicals and cancer, as they exhibit the capacity to regulate cell

cycle dynamics, curb tumor cell proliferation, and influence tumorigenesis through their involvement in diverse signaling pathways [13], [14].

In order target this pathway, we performed this study utilizing computational approaches to explore novel compounds and repurpose existing ones. The study performed aims to screen and evaluate compounds that have the potential to bind to and inhibit STAT3 and STAT1 thereby disrupting its signaling pathway providing new treatment options for GBM. To uncover potential compounds, we leveraged phytochemicals sourced from Indian medicinal plants and FDA approved drugs renowned for their diverse therapeutic attributes. These agents not only endorse novel and alternative avenues for cancer chemotherapy and may also enhance the therapeutic efficacy of of a diverse range of medications.

1.2. Glioblastoma:

1.2.1 Basic Outline

Gliomas are infiltrative tumors and represents different types of primary malignant neoplasms of CNS, originating from glial cells or cancer stem cells. Among these, GBM or glioblastoma is most malignant, accounting for about 60-70% of gliomas. Designated as a grade IV tumor by the WHO, GBM stands as the predominant brain neoplasm affecting various age groups. GBM is categorized into three major subtypes based on histopathology and into four major sub-types according to gene expression profiles. Additionally, it is further subdivided according to the mutational status of the IDH (isocitrate dehydrogenase) genes [15], [16]. Although the incidence of glioblastoma is relatively low, affecting 3.19 people per 100,000, its extremely low survival rate has garnered significant research interest. Only 5% of those diagnosed with glioblastoma are expected to survive for at least five years [17]. The recurrence of GBM, despite advances in treatment, is driven by its resistance to diverse therapies, the constraints of surgical resection, and the absence of targeted interventions. These factors collectively contribute to the decrease in survival rates observed in patients [18], [19].

1.2.2 Clinical Presentation and Distribution of Glioblastoma

A comprehensive clinical history is essential for GBM patients as their symptoms mimic those of benign or malignant brain tumors. Typically, these neurological symptoms develop progressively over a period of time, requiring a high level of suspicion for GBM due to symptom overlap with infectious or inflammatory conditions [20].

GBM predominantly occurs in the supratentorial region, accounting for 85% of cases. In contrast, it is found in the brainstem and spinal cord in less than 5% of cases, with the brainstem being the least affected at under 3%. In the context of glioblastoma cohorts, the frequency and patterns of clinical presentations include intracranial

hypertension in 30% of cases, motor deficits and epilepsy each in 20%, altered sensorium in 17%, confusion manifests in 15%. Additionally, both visual and speech deficits are identified in 13% of cases within these cohorts [21].

1.2.3 Histopathology & Pathphysiology

Histologically, GBM is distinguished by small, polygonal to spindle-shaped cells with acidic cytoplasm and vague cellular boundaries. The nuclei, distinguished by their oval or elongated forms, exhibit aggregated hyper-chromatic chromatin and multiple distinct nucleoli, along with significant nuclear pleomorphism. The presence of intranuclear inclusions with binuclear and multinucleated cells characterizes this malignancy [22]. During vascularization, GBM forms new vessels that resemble renal glomeruli, with endothelial cells that are phenotypically distinct, exhibiting focal overlap, hyperplasia and heterogeneity in size and shape. The newly developed vessels oftenly have numerous Weibel-Palade bodies and thrombi, while also exhibiting two distinct patterns of necrotic areas [23], [24]. Glioblastoma is marked by undifferentiated cells, pronounced cellular variability and shows elevated mitotic activity, extensive vascularization, immune escape, marked tumor heterogeneity, significant local invasiveness and necrosis [24], [25].

In addition to these factors, the immune-privileged environment within the CNS, devoid of antigen-presenting cells and lymphatics, worsens its the prognosis. In GBM, malignant cells display angiogenesis, abberant proliferation and rapid growth driven by genetic and epigenetic mutations, crucial for understanding tumor dynamics and treatment resistance. GBM is categorized into primary and secondary tumors depending on whether the initiating mutations occur in stem cells or mature neural cells, respectively. Genetic alterations induce varied biochemical shifts, including gene suppression or over-expression compared to healthy brain cells, resulting in cellular and extracellular matrix alterations that contribute multiform nature of the disease [26], [27], [28], [29].

1.2.4 Treatment Strategies

The infiltrative and intricate nature of GBM along with resistant glioblastoma stem cells, tumor heterogeneity and the formidable BBB, presents significant challenges in treatment, highlighting the urgent need for innovative therapeutic approaches [30], [31].

The prevailing gold standard for GBM treatment comprises surgical resection, succeeded by radiotherapy and temozolomide chemotherapy, which can extend survival in younger patients up to 202 weeks [32], [33]. This multimodal anti-cancer strategy, integrating surgery, radiotherapy, and chemotherapy aims to achieve tumor regression and maximize disease-free survival. Additionally, anti-angiogenic gene therapy targeting the VEGF-dependent pathway, adjuvant therapies to enhance short term survival and hormone treatments inhibiting the JNK-dependent signaling

pathway offer promising adjuncts. Bevacizumab, a humanized IgG1 monoclonal antibody, has demonstrated significant anti-GBM efficacy, improving patient outcomes in combination with chemotherapy, and has received accelerated approval for recurrent GBM [34], [35], [36].

Emerging therapeutic strategies include cancer vaccines, immune checkpoint inhibitors, CAR T-cell therapy and viruses involved in oncolysis, all showing potential to enhance GBM patient survival [37], [38]. Despite the development and ongoing refinement of multiple therapies, a comprehensive and universally effective treatment approach remains to be fully realized, necessitating continued research and innovation in GBM treatment modalities.

1.3. JAK-STAT Signalling Pathway

1.3.1 General Review

The JAK/STAT signaling is a pivotal cellular communication cascade regulating diverse downstream processes in response to various cytokines and growth factors [39], [40], [41]. In addition to regulating gene expression, this pathway governs cell proliferation, differentiation, activation, autophagy and apoptosis [42], [43]. It plays a crucial role in regulating growth, hormonal release, tumor progression and inflammation. Any impairment in this pathway can lead to a range of illnesses, such as cancer, inflammatory and neurodegenerative disorders [44], [45].

1.3.2 Components

This evolutionary conserved cascade structurally contains trans-membrane receptors, receptor associated JAKs and STATs. The JAK family encompasses four proteins whereas the STAT family includes seven distinct proteins [46].

JAKs are tyrosine kinases linked to ligand-receptor complexes, activated by growth factors and cytokines, composed of four domains. The FERM and SH2 domains facilitate JAK binding to receptors, while the pseudo-kinase domain modulates kinase domain activity essential for receptor tyrosine phosphorylation [47], [48], [49]. STATs are downstream signaling molecules featuring six structurally conserved domains. At either end there are N-terminal domain and transcription-activation domain (TAD) interspersed with coiled-coil domain (CCD), DNA-binding domain (DBD), connection/linker domain and SH2 domain [50].

STAT homodimerization and nuclear transfer is facilitated by N-terminal domain which operates independently, even in unphosphorylated state [51], [52], [53]. The coiled-coil domain composed of multiple alpha-helices, interacts to transcription factors and co-activators and contributes in nuclear translocation [54], [55], [56]. The DBD is is essential for recognizing and binding to regulatory sequences of target

genes as well as regulating nuclear import and export [57]. The linker domain links DBD to SH2 domain, a highly conserved structure binding to specific phosphotyrosine motifs at the activated receptor complex and facilitates protein-protein interactions [58], [59], [60]. The TAD at the C- terminus contains phosphorylation sites critical for STAT activation and can prevent auto-phosphorylation [61].

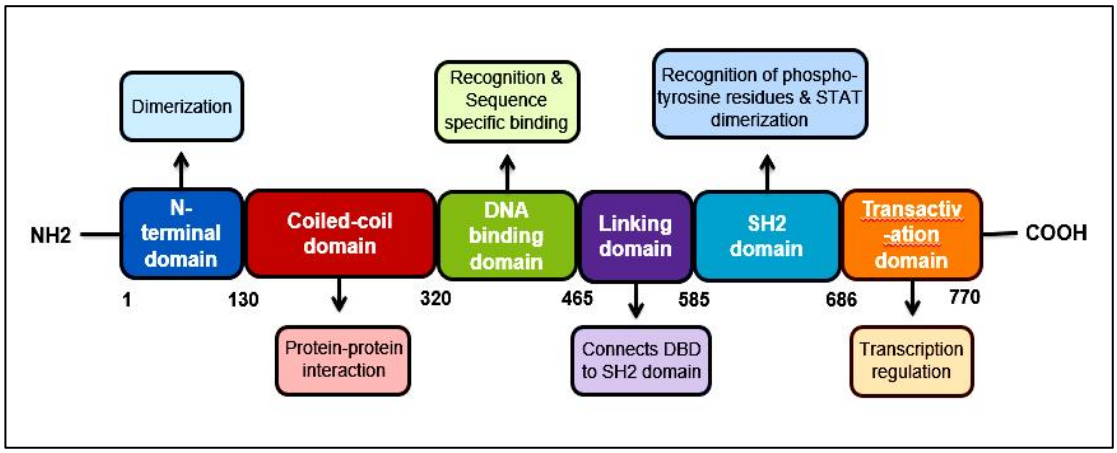


Fig. 1.1 Comprehensive representation of STAT3 protein domains and their functional roles.

1.3.3 Physiology

JAK-STAT Pathway activation commences with the binding of ligands, leading a structural change in the receptor that promotes dimerization. This event facilitates the recruitment and binding of JAK proteins to specific sites on the receptor. Subsequent non-covalent binding of STAT proteins via their Src homology domains which mediates phosphorylation of tyrosine residues of the receptor. Once bound, STAT proteins become activated, forming dimers or even tetramers. These activated STATs complexes translaocate to the nucleus to function as transcription factors, thereby modulating gene expression [62], [63], [64], [65].

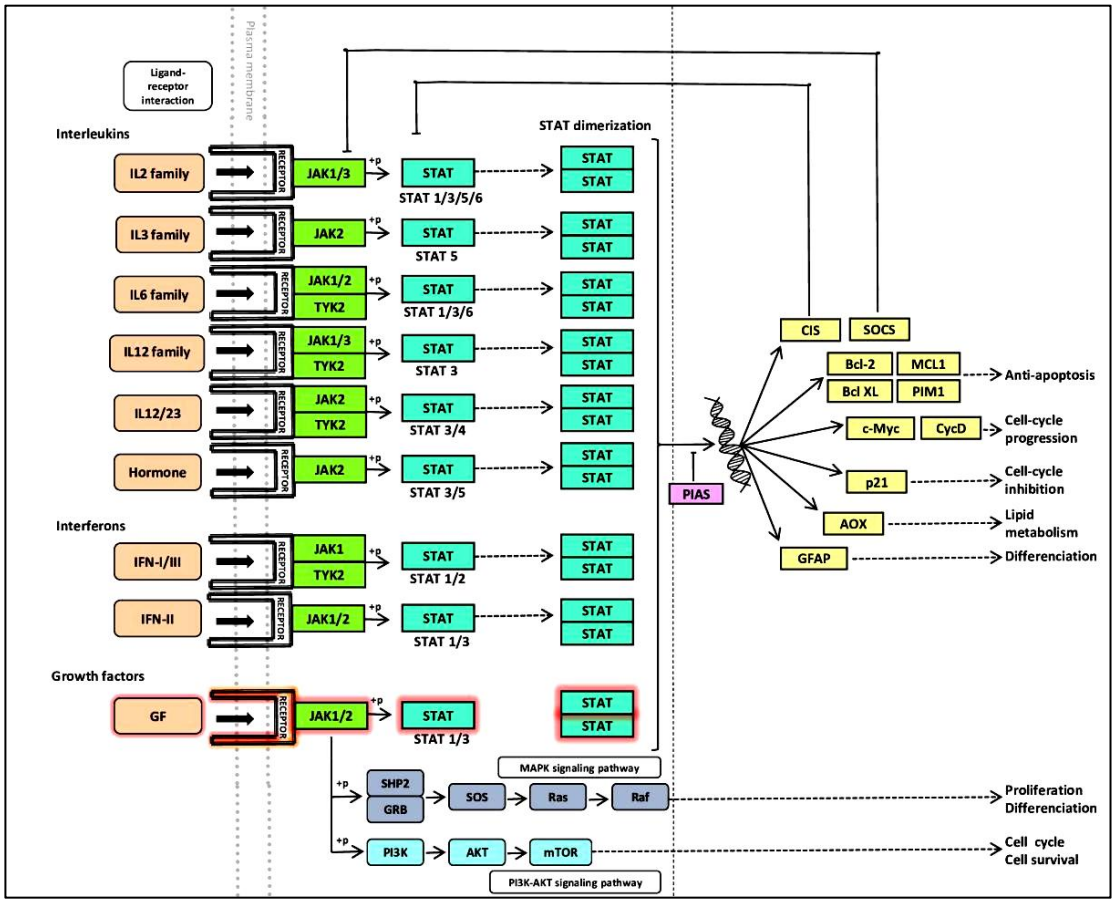


Fig. 1.2 JAK/STAT Signaling Pathway

1.3.4 STAT3 and GBM

Out of other STAT protein, STAT3 a pivotal role in various biological functions. It regulates factors critical in cancer processes such as cell growth, apoptosis, cancer stemness, immune regulation, metastasis and tumorigenesis [66], [67], [68], [69]. Its involvement in these processes and the pathogenesis of multiple malignancies, particularly in the invasive growth of gliomas, makes it a promising therapeutic target [70], [71]. Research indicates that phosphorylated STAT3 (p-STAT3) is implicated in invasion and metastasis and is present in approximately 60% of human GBM cohorts [72]. In GBM, STAT3 activation is linked to several regulators, including BMX (bone marrow kinase X), which is upregulated in GBM stem cells and epidermal growth factor which results in imbalancing of STAT3 pathway [73] [74]. The GBM microenvironment, characterized by high levels of IL-6 and other inflammatory factors, directly activates STAT3, thereby promoting GBM growth and migration. STAT3 contributes to invasion by upregulation of pro-invasive factors and facilitating the development of aggressive phenotype through interaction with VEGF and HIF-1[75], [76]. Aberrant STAT3 activation is associated with increased recurrence rates, invasiveness, and migration activity following radiotherapy due to the activation of downstream signaling pathways [77], [78]. In-vitro settings have

shown that STAT3 inhibitors exert inhibitory effects on GBM cells. However, many known STAT3 inhibitors, such as ursolic acid, face challenges including high toxicity, impermeability to the blood-brain barrier and rapid metabolization, rendering them unsuitable for clinical application in GBM therapy [79], [80]. A recent study demonstrated that the synergy of STAT3 inhibition and radiation therapy reprogrammed the tumor micro-environment immunologically, markedly enhancing animal survival and and highlighting the critical role of an intact immune response for the efficacy of STAT3 inhibition [81].

As STAT3 is known to exhibit progression of GBM, targeting STAT3 could be an effective therapeutic approach. Combining STAT3 inhibitors with conventional therapies may enhance the current treatment outcomes for GBM.

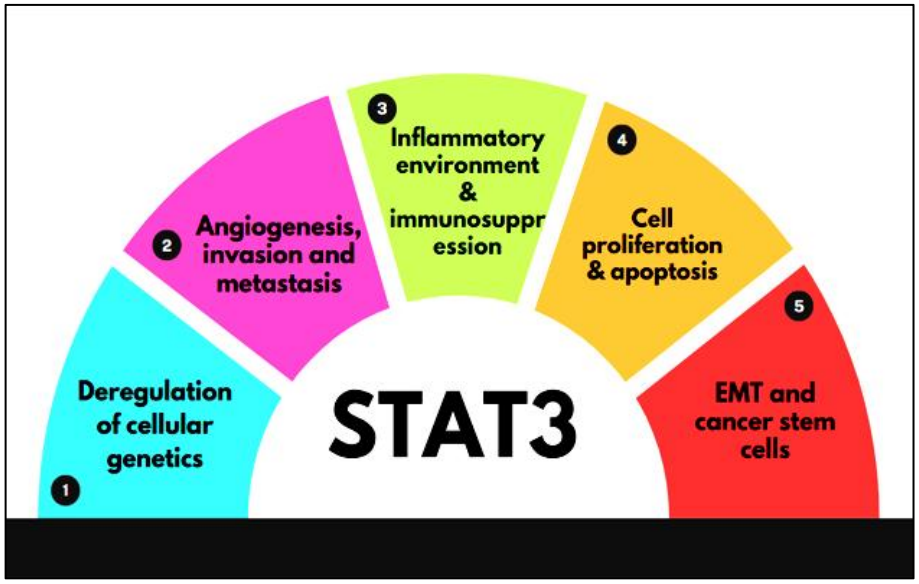


Fig. 1.3 Illustration of STAT3 pivotal role with diverse tumor hallmarks

1.3.5 STAT1 and GBM

STAT1, the first discovered transcription factor of the STAT family plays role in IFN signaling [82], [83], [84]. STAT1 is integral to numerous functions, such as cell growth, proliferation and tumor development [85]. In gliomas role of STAT1 is intricate. Previous STAT1 has been recognized for its tumor-suppressive functions with studies indicating that STAT1 upregulation leads to reduced glioma cell proliferation and migration while promoting apoptosis [86], [87]. However, recent research has revealed contradictory findings. For instance, IGFBP-3 is involved in the pathogenesis of GBM, induces STAT1 overexpression in glioma cells. Additionally, IL-8 in the GBM microenvironment facilitates mesenchymal transition, migration and invasion via the STAT1/HIF-1α/Snail pathway and IFN-γ signaling which enhances GBM cell migration, also elevates STAT1 levels [88], [89], [90].

A study highlights that STAT1 expression is linked to GBM aggressiveness and its down-regulation mitigates this aggressiveness, suggesting STAT1 as a potential therapeutic target against GBM [91].

CHAPTER - 2

METHODOLOGY

2.1. Sources

Databases: PubMed, PubChem, IMPAAT 2.0, PubChem, Protein Data Bank (PDB), UniProt
Software: Open Babel, PyRx, CB-Dock2, Discovery Studio, PyMOL

2.2. Workflow

From literature review, STAT3 was recognised an potential target for the discovery of novel phytochemicals having the inhibitory effect, for targeting the JAK/STAT cascade in GBM. For identification of ligands molecules, the plants were selected with multiple therapeutic applications. The structure of plant specific phytochemicals was retrieved from IMPPAT 2.0 and then filtered out based on blood-brain barrier permeability (BBB). Alongside 3,674 FDA approved drugs were also taken. These selected candidates were considered as ligands for the further investigation. The workflow sequential process in Fig. 2.1.

2.3. Molecular docking

2.3.1 IMPPAT 2.0

IMPPAT 2.0 stands as the most comprehensive manually curated database of phytochemicals, compiled by digitizing extensive data on traditional Indian medicinal plants. This platform highlights the connections between plants, their respective parts, phytochemicals and therapeutic applications. This integrated resource, IMPPAT 2.0 underscores the knowledge inherent in traditional Indian medicine and supports natural product-based drug discovery.

2.3.2 Data Extraction

For ligand data extraction, 30 Indian medicinal plants with wide range of activities such as anticancer, anti-inflammatory, antimalarial, anti-allergic, anti-diabetic, anti-nociceptive, analgesic, antimicrobial, cytotoxic, antioxidant, antilipidimic, hepatoprotective, vasorelaxant, antitumor, neuroprotective, antibacterial, anti-proliferative, antifungal, antiulcer, anti-diarrhoeal, immunomodulatory, anti-pyretic, antiplasmodic, antihistaminic, antihelmintic, astringent, anti-hyperglycaemic, anti-spasmodic and others mentioned in the table 2.1 were selected. The 3D .pdb structure of phytochemicals specific for each plant was downloaded from IMPAAT 2.0 by making individual entry. The structure of reference compound, curcumin identified

by literature survey were retrieved using IMPAAT 2.0 of ID IMPHY007574 in 3D .sdf format. For FDA-approved drugs, the compounds were downloaded from DrugBank, using napabucasin as the reference drug with its structure obtained from PubChem. The target protein STAT3 is downloaded from Protein Data Bank (PDB) in .pdb format.

Table 2.1 List of phytocompounds derived from IMPPAT 2.0

Name of medicinal plant	**Family**	**Number of entries**	**Source of phytochemicals**	**Activity**	**Reference**
Albizia lebbeck	Fabaceae	108	Bark, flower, fruit, leaf, root, seed, wood	Anticancer, anti-nociceptive, anti-inflammatory, antimalarial, anti-allergic, neuroprotective	[92], [93], [94], [95], [96]
Anona squamosa	Annonaceae	441	Bark, rooot, fruit, leaf, seed, stem, whole plant	Analgesic, anti-inflammatory, antimicrobial, antioxidant, antilipidimic, hepatoprotective, vasorelaxant, antitumor	[97]
Arnebia euchroma	Boraginaceae	29	Plant cells/culture, root	Anti-bacterial, anti-proliferative, anti-inflammatory, antioxidant	[98], [99], [100]
Asparagus officinalis	Asparagaceae	51	Flower, leaf, root, seed, shoot	Anti-diabetic, anticancer, antifungal, antimicrobial	[101], [102], [103]
Asparagus racemosus	Liliaceae	40	Bark, flower, fruit, leaf, root, wood,	Antiulcer, antioxidant, antidiarrhoeal, antidiabetic, immunomodulatory, antitumor	[104], [105]
Bauhinia racemosa	Fabaceae	20	Bark, root, seed, stem, wood	Analgesic, antipyretic, anti–inflammatory, anti-plasmodic, antimicrobial, antihistaminic	[106], [107], [108], [109]
Bidens pilosa	Asteraceae	190	Flower, leaf, root, stem	Anti-proliferative, anti-inflammatory, anti-diabetic, antioxidant, antimalarial	[110], [111], [112], [113]
Butea monosperma	Fabaceae	77	Bark, flower, plant exudate, root, seed, whole	Antitumor, antimicrobial, anti-helmintic,	[114], [115]

			plant	anti-inflammatory, astringent	
Calotropis gigantea	Asclepiadaceae	89	Aerial part, bark, flower, leaf, plant exudate, root, seed, stem	Anti-inflammatory, antioxidant, anticancer	[116], [117]
Cardiospermum halicacabum	Sapindaceae	32	Leaf, root, seed	Antipyretic, antimalarial, antioxidant, antiulcer, anti-hyperglycaemic, antispasmodic, antitumor	[118], [119], [120]
Cedrus deodara	Pinaceae	189	Bark, flower, leaf, plant exudate, root, seed, wood, whole plant	Anti-inflammatory, anti-hyperglycaemic, antimicrobial, anti-apoptotic, immunomodulatory, antimalarial, antiulcer, anticancer, analgesic	[121]
Centella asiatica	Apiaceae	97	Aerial part, leaf, whole plant	anti-inflammatory, antipsoriatic, antiulcer, immunostimulant, cardioprotective, antitumor, antiviral, antioxidant	[122], [123], [124], [125], [126], [127], [128]
Cinnamomum zeylanicum	Lauraceae	515	Aerial part, bark, leaf, fruit,	anti-inflammatory, anti-microbial, cardio-protective	[129]
Clerodendrum glandulosum	Lamiaceae	9	Leaf, root	antioxidant, hepatoprotective	[130], [131]
Croton tiglium	Euphorbiaceae	33	Seed	Antibacterial, antifungal, analgesic, anti-inflammatory, anti-HIV, antitumor	[132], [133], [134], [135]
Datura innoxia	Solanaceae	53	Aerial part, flower, fruit, leaf, root, seed, stem	Analgesic, anthelmintic, anti-inflammatory	[136]
Datura metel	Solanaceae	104	Aerial, leaf, part, stem, bark, root, flower, fruit, seed, whole plant	Anti-proliferative, anti-inflammatory, antioxidant, antipyretic, analgesic	[137], [138], [139], [140],
Euphorbia hirta	Euphorbiaceae	129	Aerial, part, bark, flower, leaf, plant exudate, root, stem, whole plant	Anthelmintic, antimicrobial, antimalarial, antispasmodic	[141]
Gymnema	Asclepiadacea	119	Fruit, leaf	Antioxidant,	[142],

sylvestre	e			anti-diabetic, antimicrobial, anti-inflammatory, anticancer	[143]
Inula racemosa	Compositae	48	Root	Anti-inflammatory, analgesic, anticancer	[144]
Magnolia grandiflora	Magnoliaceae	240	Bark, flower, leaf, seed, stem, wood	Antimicrobial, anticonvulsant, muscle relaxant, anti-inflammatory, analgesic	[145], [146], [147], [148]
Moringa oliefera	Moringaceae	200	Bark, stem, flower, root, fruit, leaf, seed, whole plant	Antioxidant, anticancer, anti-inflammatory	[149]
Plantago major	Plantaginaceae	46	Aerial part, flower, leaf, root, seed, whole plant	Hepatoprotective, anti-hypercholesteremia, anti-atherosclerosis, anti-inflammatory, analgesic, antimicrobial, anticancer	[150], [151], [152]
Pterocarpus marsupium	Fabaceae	71	Bark, root, seed, whole plant, wood	Antihelminthic, antipyretic, anti-inflammatory, aphrodisiac, antiulcer	[153]
Semecarpus anacardium	Anacardiaceae	35	Fruit, leaf, seed, whole plant	anti-atherogenic, anti-inflammatory, antioxidant, anti-reproductive, anti-carcinogenic central nervous system stimulating, hypoglycemic,	[154]
Taxus wallichiana	Taxaceae	181	Bark, fruit, leaf, Root, stem, wood	Analgesic, anti-inflammatory, immunomodulatory, antispasmodic, antiallergic, anticonvulsant, anti-osteoporotic, anti-cociceptive	[155], [156]
Urtica dioica	Urticaceae	69	Flower, leaf, plant cells/culture, rhizome, root, trichome	Antioxidant, anti-inflammatory, hypoglycemic, antiulcer, cardiovascular protective, repression of prostate-cell metabolism, proliferation	[157], [158]
Vernonia	Asteraceae	57	Aerial part,	Antimicrobial,	[159],

cinerea			flower, leaf, root, seed, whole plant	antipyretic, anti-helmentic, anti-inflammatory, analgesic	[160]
Vitex negundo	Verbenaceae	228	Bark, flower, fruit, leaf, root, seed, stem	Antihelmintic, anti-inflammatory, anti-proliferative, antioxidant	[161], [162]
Withania somnifera	Solanaceae	129	Leaf, root, seed	Anticancer, anti-inflammatory, antioxidant	[163], [164]

2.3.3 Ligand Preparation

For preparation of ligands, the downloaded compounds were converted from .pdb file to SMILE files using a versatile and open source toolbox, Open Babel. Then all these compounds were loaded in Swiss ADME to check BBB permeability. The data obtained from Swiss ADME was analyzed manually and the BBB permeable compounds were considered as ligands and used for docking purpose. In case of FDA approved, once the structure of drugs were obtained, Open Babel was used to convert the retrieved compounds into .sdf format for docking.

2.3.4 Protein Preparation

For preparation of protein, protein structure having PDB ID 6TLC with resolution of 2.90 Å is retrieved from PDB. PyMol was used to open the downloaded .pdb file and the protein is checked for errors and corrections were made, in which the mono-bodies and extra-chain was removed. Furthermore, the water molecules are removed and the gaps will filled, modified STAT3 protein structure was saved in .pdb format.

2.3.5 PyRx

PyRx, an open source software is used for virtual screening of libraries for identification of potential drug targets. It is a substantial collection of various software which makes it a valuable asset for computer aided drug discovery. For the study performed Open Babel is used for importing ligand files and vina wizard for the docking purpose.

2.3.6 Docking

Docking is performed after preparation of ligands and protein. The .sdf file of ligands and the protein structure was uploaded in PyRx. The inbuilt Open Babel converts the ligands to .pdbqt format after energy minimization. The protein molecule is first loaded and then converted to macromolecule. Subsequently, the ligands and protein were chosen, and the grid-box dimensions were adjusted to ensure the entire protein was encompassed within the grid box after utilizing the forward option. Once the docking is completed the results were saved as output files and .csv files which were further analyzed.

2.4. Docking Analysis

For the selection of efficient ligands .csv files for each plant were analyzed and the potential ligands were identified based on the docking score. To identify efficient FDA-approved drugs, the drugs with highest affinity were first selected by analyzing .csv files. Among these, the drugs that are permeable to the blood-brain barrier (BBB) were then identified and subjected to further analysis.

2.5. Docking Result Validation

To validate the results CB-Dock2 was employed, an advanced blind docking server designed for virtual screening.

2.6. Protein-Ligand Interaction Visualization

The output files of the selected ligands were analyzed using Discovery Studio platform and PyMOL for 2D and 3D interactions with the the protein. These platforms enable the visualization of ligand binding sites on proteins, detailing the number of interacting residues, as well as identifying the specific amino acids involved in these interactions.

2.7. ADME Analysis

The physiochemical, drug likeliness and pharmacokinetic properties of hit compounds were evaluated by Swiss ADME, a web tool that offers free access to quick and reliable predictive models. It involves analysis of BBB permeability, total molecular weight, rotatable bonds, hydrogen acceptor, hydrogen donor, topological polar surface area, lipophilicity, GI absorption, cytochrome P450 enzyme inhibition, solubility, bioavailability and lipinski's rule of 5.

2.8. Toxicity and Carcinogenicity Analysis

For evaluation of toxicity, another freely available web tool pkCSM used for making rapid pharmacokinetic properties prediction. For the study, it was used for determining the oral rat acute toxicity LD50 and max. human tolerated dose. For carcinogenicity testing, the web-based tool CarcinoPred-EL was utilized. This advanced platform integrates three novel ensemble learning models to predict whether compounds are carcinogenic or non-carcinogenic. Therefore, various properties of the hit compounds were analyzed just by input of SMILES extracted from IMPPAT 2.0 in each tool.

2.9. In-silico based Cancer Cell Cytotoxicity Analysis

CLC-Pred (Cell Line Cytotoxicity Predictor) 2.0 is an effective web tool which mediates in-silico prediction of cytotoxic effect of chemical and natural compounds against hundreds of cancer cell lines and normal cell lines. It involves use of PASS technology and make predictions based on the structural formula. For this analysis, we input the SMILES representations of the lead compounds into CLC-Pred 2.0

[165]. The results obtained includes cytotoxicity data against various tumor types, but we focused specifically on the cytotoxicity against gliomas and glioblastoma and cytotoxicity data was recorded in terms of Pa > Pi values.

2.10. STAT1 docking with lead compounds

Following multiple analysis, the identified hit compounds were docked to the STAT1 (1YVL) protein, whose structure was obtained from the PDB. The protein was prepared similarly to STAT3 and docking was performed using PyRX.

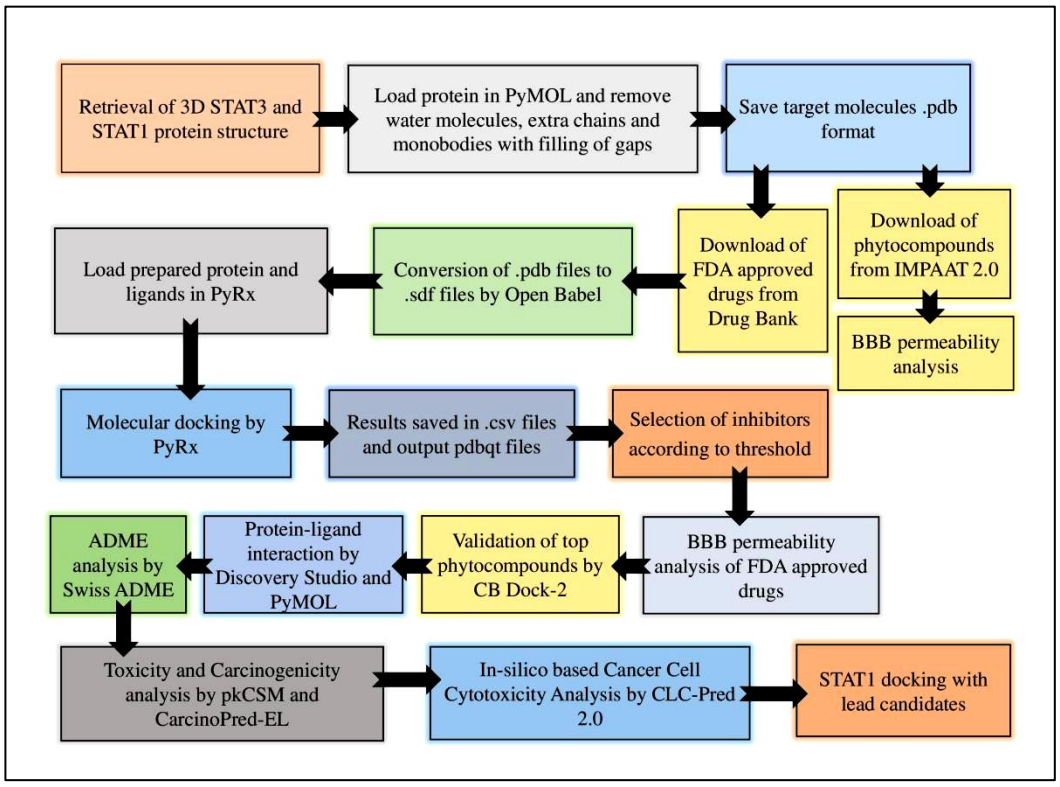


Fig. 2.1 Overview of performed methodology

CHAPTER - 3

RESULT AND DISCUSSION

3.1. Result

3.1.1 Docking Result

The blood brain permeable phtyochemicals derived from 30 Indian medicinal plants undergoes molecular docking along with the reference compound curcumin. The docking score of 6.5 kcal/mol was obtained for the reference compound with STAT3. For identification of effective STAT3 inhibitor the compounds with binding affinity of 8.1 kacl/mol or lower were considered. Among the phytochemicals sourced from 30 different plants, only those from 9 plants met the desired criteria outlined in the **t**able 3.1. The phytochemical with the most negative docking score, indicating the most stable ligand-protein complex, was derived from the Datura metel plant with an IMPAAAT ID, IMPHY003277. This compound emerged as the most potent inhibitor, boasting a docking score of -8.9 kcal/mol.

Table 3.1 Binding affinities of compounds within threshold range

Medicinal plant	**IMPPAT ID**	**Phytochemicals**	**Binding Affinity**
Anona squamosa	IMPHY005339	Anolobine	-8.7
	IMPHY001483	Xylopine	-8.5
	IMPHY001885	Annosquamosin D	-8.4
	IMPHY001469	Anonaine	-8.4
	IMPHY000175	Annosquamosin B	-8.3
	IMPHY001853	Aporphine	-8.2
	IMPHY005314	Norlaureline	-8.2
	IMPHY002753	Liriodenine	-8.1
	IMPHY013006	Michelalbine	-8
Arnebia euchroma	IMPHY007551	Deoxyshikonin	-8.2
Asparagus officinalis	IMPHY012274	Sarsasapogenin	-8.1
	IMPHY003711	Yamogenin	-8.1
Calotropis gigentea	IMPHY003772	Uzarigenin	-8.1
Datura metel	IMPHY003277	Withametelin	-8.9
	IMPHY010687	Isowithametelin	-8.8
	IMPHY004278	Datumetelin	-8.2
Magnolia grandiflora	IMPHY005339	Anolobine	-8.7
	IMPHY001469	Anonaine	-8.4
	IMPHY002753	Liriodenine	-8.2
	IMPHY014895	Sesamin	-8
Pterocarpus marsupium	IMPHY001869	Liquiritigenin	-8.2

Utrica diocia	IMPHY002029	Luteoxanthin	-8.4
Withania somnifera	IMPHY000630	Withasomidienone	-8.6
	IMPHY004033	Solasodine	-8.1

For FDA-approved drugs, elbasvir showed the most negative binding energy score of -10 kcal/mol, while the reference compound scored -7.5 kcal/mol. A threshold of ≤ -8.5 kcal/mol was set based on the number of drugs with high affinity. Compounds within this range were then analyzed for BBB permeability using Swiss ADME. Ten compounds met the criteria, with the top three drugs tirilazad, telmisartan and mizolastine with binding affinity -9.5 kcal/mol, -9.1 kcal/mol and -9 kcal/mol were selected for further investigation and mentioned in Table 3.2.

Table 3.2 Leading FDA drugs exhibiting best affinity

S. No.	FDA Approved Drug	Binding Affinity (kcal/mol)
1.	Tirilazad	-9.5
2.	Telmisartan	-9.1
3.	Mizolastine	-9

3.1.2 Validation Result

Upon validating the top five phytocompounds and top three drug candidates with CB-Dock2, notably significant values were obtained, with differences between the scores from PyRx and CB-Dock2 being approximately ≤ 0.5 kcal/mol. The docking score of the reference compound, curcumin was -7.3 kcal/moland napabucasin was -8.1 kcal/mol for curcumin. The docking scores of these compounds were mentioned in the Table 3.3 and 3.4.

Table 3.3 Comparison of phytocompound binding scores from PyRx and CB-Dock2

Target Protein	IMPPAT ID	PyRx	CB-Dock 2
STAT3	IMPHY003277	-8.9	-9
	IMPHY010687	-8.8	-9.3
	IMPHY005339	-8.7	-8.7
	IMPHY000630	-8.6	-9
	IMPHY001483	-8.5	-8.7

Table 3.4 Comparison of FDA approved drugs binding scores from PyRx and CB-Dock2

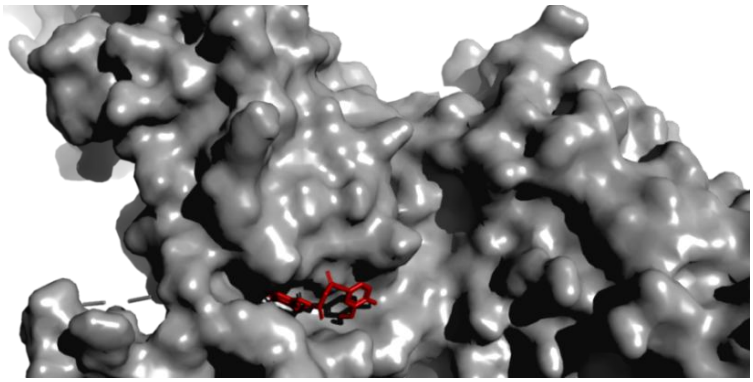
Target Protein	FDA Drug	PyRx	CB-Dock 2
STAT3	Tirilazad	-9.5	-9.2
	Telmisartan	-9.1	-9.0
	Mizolastine	-9	-8.5

3.1.3 Protein-Ligand Interaction Result

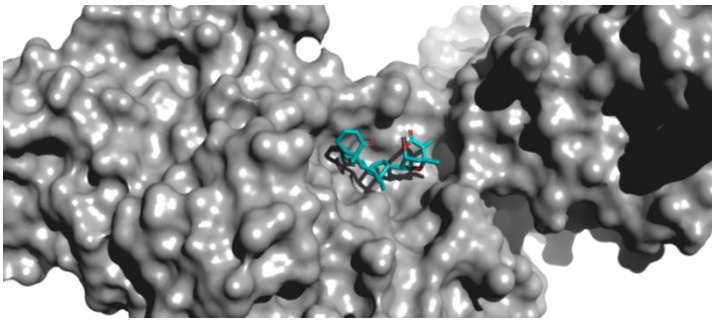
The 2D and 3D interactions of the top five phytocompounds and best three drug candidates with STAT3 were analyzed using Discovery Studio and PyMOL, utilizing output files from PyRx as detailed below. Furthermore, interaction of reference compounds are also included.

Table 3.5 Interacting residues of STAT3 with leading phytocompounds and reference compound

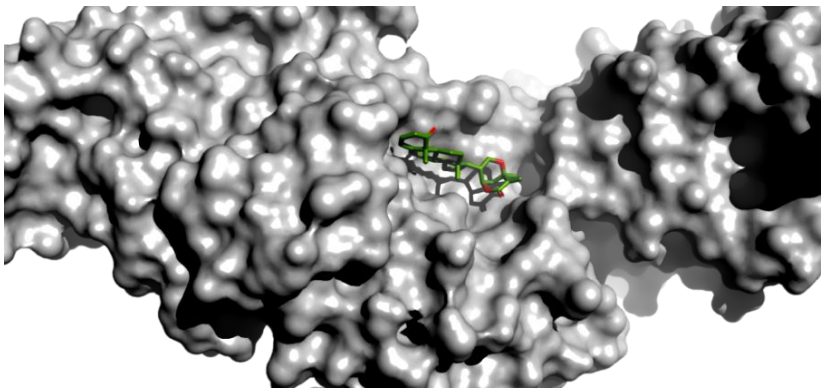
S.No.	IMPPAT ID	Interacting Residues
1.	IMPHY003277	Arg325, Gln326, Pro256, Ser514, Gly253, Asp334, Pro333, Pro336, Ala250, Cys251, Gln247, Glu324, Trp243, Asn257, Leu260, Cys259
2.	IMPHY010687	Asp334, Cys251, Ser514, Ala250, Gln247, Asn257, Trp243, Glu324, Leu260, Cys259, Arg350, Arg325, Pro336, Pro333
3.	IMPHY005339	Leu 438, Val490, Arg397, Ser381, Glu435, Leu436, His437, Asp369, Lys370, Thr440
4.	IMPHY000630	Arg325, Gly253, Asp334, Thr515, Ser514, Pro333, Pro336, Gln326, Gln247, Ala250, Asn257, Cy251, Trp243, Glu324, Leu260, Cys259
5.	IMPHY001483	Asp371, Leu438, Thr440, Asp369, His437, Lys370, Glu435, Leu436, Ser381, Arg379, Val490
6.	Curcumin	Lys370, Thr440, His457, Glu455, Asp369, His437, Glu435, Leu436, Ser372, Val490, Arg379, Asn491, Leu438, Lys488



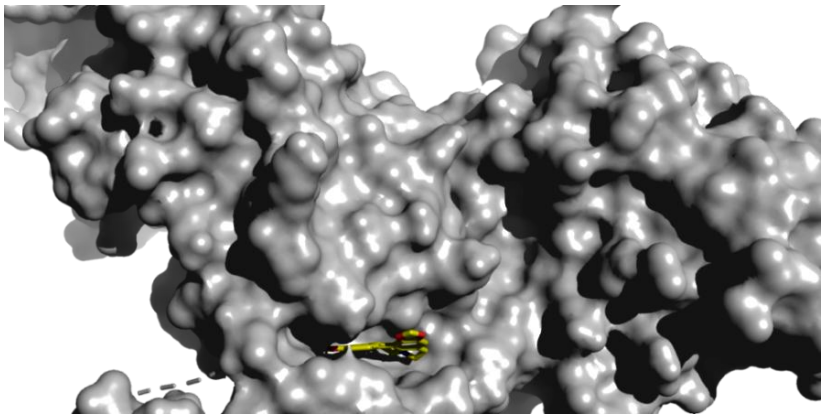
Curcumin



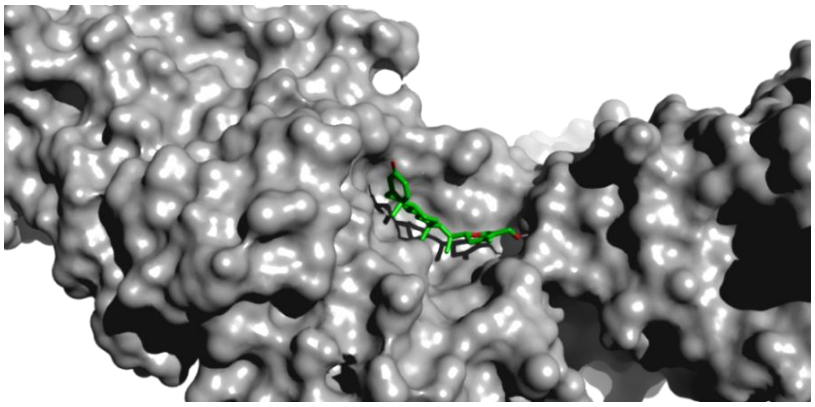
IMPHY003277



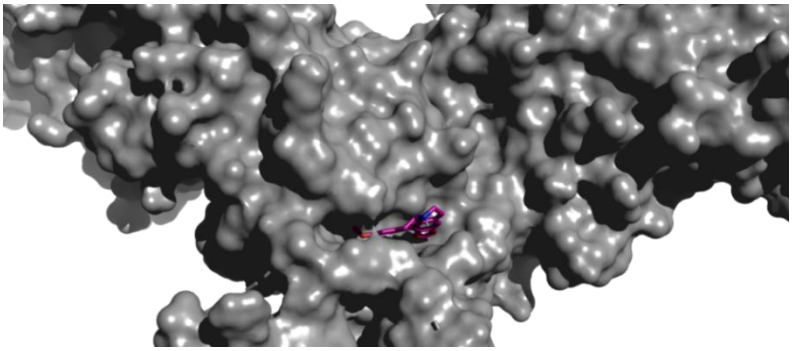
IMPHY010687



IMPHY005339



IMPHY000630

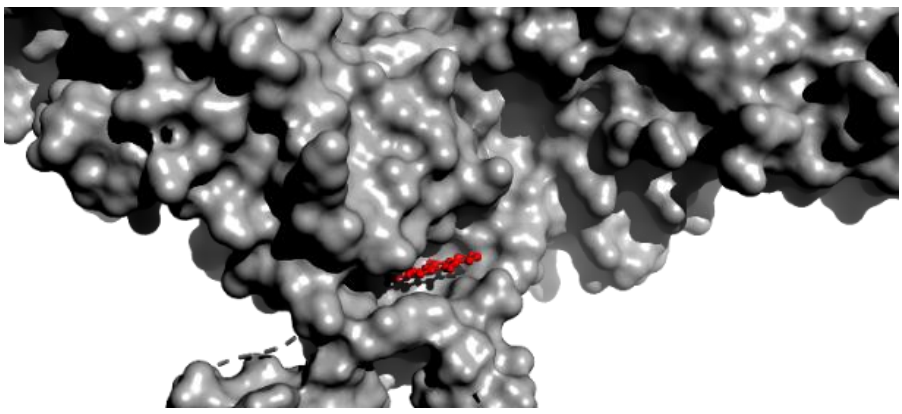


IMPHY001483

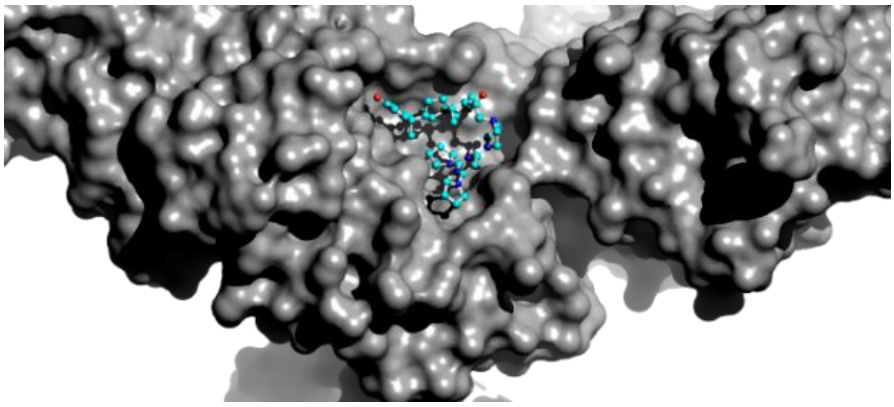
Fig. 3.1 Representation of 3D interactions between the selected reference compounds and phytocompounds with STAT3

Table 3.6 Interacting amino acids of STAT3 with leading FDA drugs and reference compound

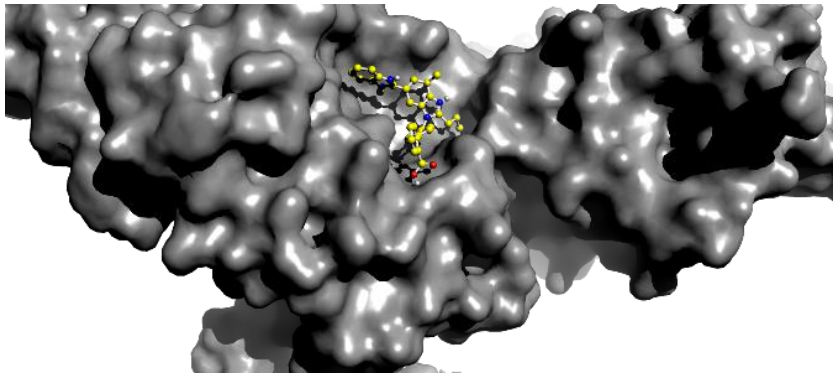
S.No.	Compound	Interacting Residues
1.	Tirilazad	Thr346, Gln326, Pro336, Arg325, Cys251, Asp334, Glu324, Cys259, Leu260, Trp243, Asn257, Gln247, Ala250, Pro256, Gly254, Pro333, Pro330, Cys328
2.	Telmisartan	Cys328, Thr346, Cys259, Leu260, Glu324, Asn257, Trp243, Gln247, Pro256, Ala250, Cys251, Gly253, Asp334, Ser514, Ile252, Pro333, Met329, Pro330, Arg325, Pro336, Trp474
3.	Mizolastine	Asn257, Pro336, Arg325, Cys328, Pro333, Asp334, Cys251, Ser514, Pro256, Glu324, Leu260, Cys259, Trp243, Gln247, Ala250, Gln326
4.	Napabucasin	Ser381, Leu438, Asp371, Thr440, Lys370, Asp369, Arg379, Val490, His437, Leu436



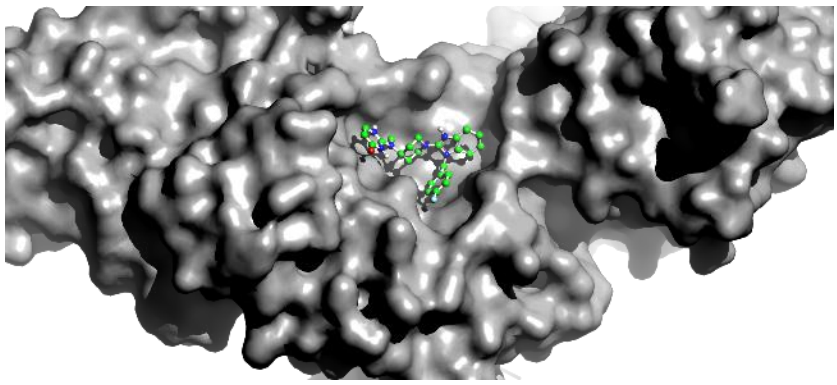
Napabucasin



Tirilazad



Telmisartan



Mizolastine

Fig. 3.2 Representation of 3D interactions between the selected reference compounds and FDA drugs with STAT3

3.1.4 ADME Analysis Result

The ADME evaluation of both the top phytocompounds and selected drugs was conducted utilizing the Swiss ADME software. The tables mentioned below presents the data on the physicochemical properties, drug-likeness, and pharmacokinetics of the identified compounds. The bioavailability radar diagrams of these phytochemicals and BIOLED-EGG images were also recorded and referenced in Fig. 3.3 and 3.4.

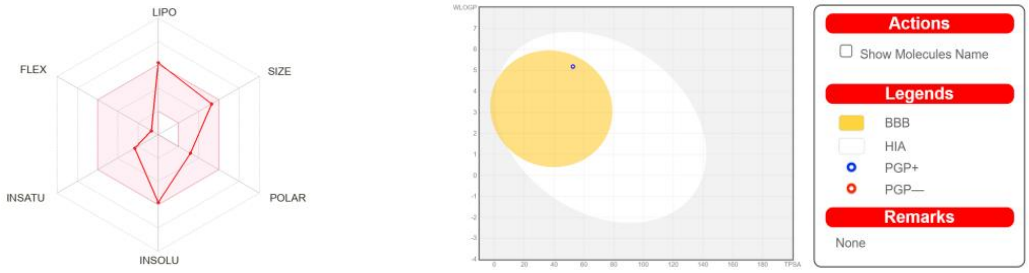
Table 3.7 Physiochemical properties exhibited by selected phytocompounds

IMPAAT ID	**Total Molecular Weight (g/mol)**	**Rotatable bonds (RB)**	**Hydrogen acceptor (HA)**	**Hydrogen donor (HD)**	**Topological Polar Surface Area (TPSA) (in Å²)**	**Consensus LogP**
IMPHY003277	436.58	1	4	0	52.60	4.72
IMPHY010687	436.59	1	4	0	52.60	4.70
IMPHY005339	281.31	0	4	2	50.72	2.47
IMPHY000630	438.60	3	4	1	63.60	4.80
IMPHY001483	295.33	1	4	1	39.72	2.88

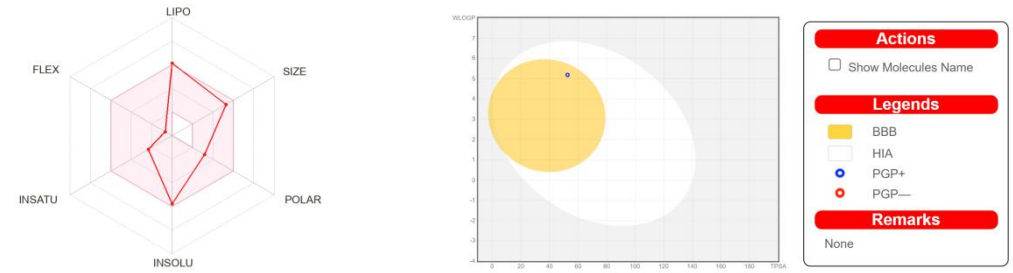
Table 3.8 & 3.9 Drug likeliness and pharmacokinetic properties of leading phytocompounds

IMPAAT ID	**GI absorption**	**CYP1A2 inhibitor**	**CYP2C19 inhibitor**	**CYP2C9 inhibitor**	**CYP2D6 inhibitor**	**CYP3A4 inhibitor**
IMPHY003277	High	No	No	Yes	No	No
IMPHY010687	High	No	No	Yes	No	No
IMPHY005339	High	Yes	No	No	Yes	Yes
IMPHY000630	High	No	No	Yes	No	No
IMPHY001483	High	Yes	Yes	No	Yes	Yes

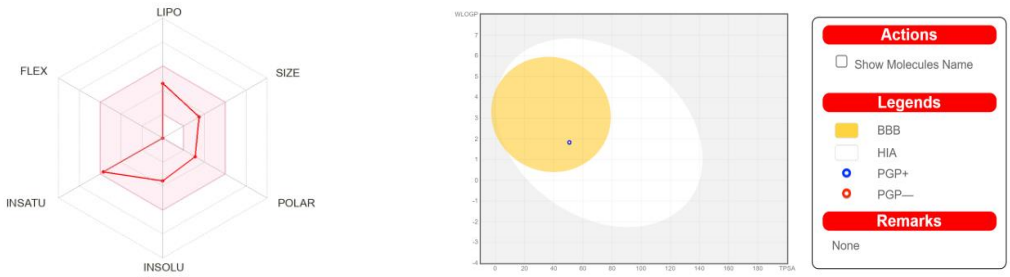
IMPAAT ID	**Solubility class**	**BBB permeant**	**Lipinski's rule of 5**	**Bioavailability score**
IMPHY003277	Moderately soluble	Yes	Passed (1 violation)	0.55
IMPHY010687	Moderately soluble	Yes	Passed (1 violation)	0.55
IMPHY005339	Moderately soluble	Yes	Passed (0 violation)	0.55
IMPHY000630	Moderately soluble	Yes	Passed (1 violation)	0.55
IMPHY001483	Moderately soluble	Yes	Passed (0 violation)	0.55



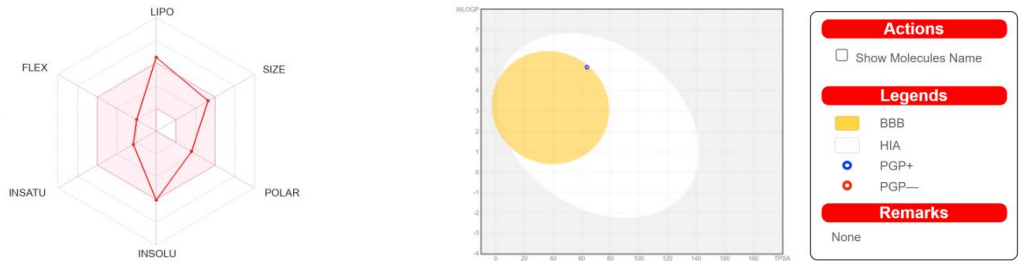
IMPHY003277



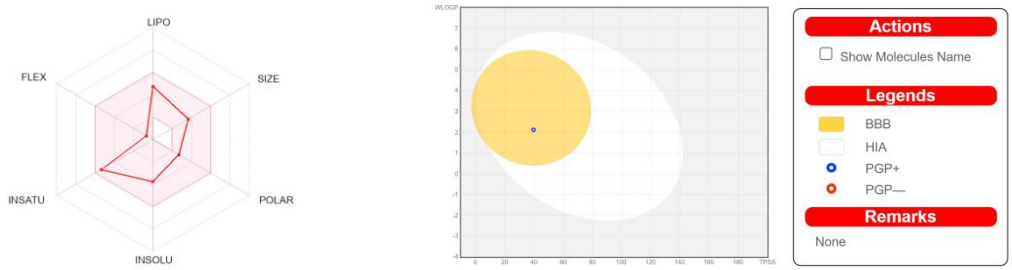
IMPHY010687



IMPHY005339



IMPHY000630



IMPHY001483

Fig. 3.3 Bioavailability radar diagrams and BOILED-EGG images of phytocompounds derived by ADME analysis

Table 3.10 Physiochemical properties exhibited by selected FDA drugs

FDA Drugs	Total Molecular Weight (g/mol)	Rotatable bonds (RB)	Hydrogen acceptor (HA)	Hydrogen donor (HD)	Topological Polar Surface Area (TPSA) (in Å²)	Consensus LogP
Tirilazad	624.86	6	5	0	72.88	5.98
Telmisartan	514.62	7	4	1	72.94	4.70
Mizolastine	432.49	5	4	1	70.05	3.28

Table 3.11 & 3.12 Drug likeliness and pharmacokinetic properties of leading FDA approved drugs

FDA Drugs	GI absorption	CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2C9 inhibitor	CYP2D6 inhibitor	CYP3A4 inhibitor
Tirilazad	High	No	No	No	No	No
Telmisartan	Low	No	Yes	No	No	Yes
Mizolastine	High	No	Yes	Yes	Yes	Yes

FDA Drugs	Solubility class	BBB permeant	Lipinski's rule of 5	Bioavailability score
Tirilazad	Poorly soluble	Yes	Passed (1 violation)	0.55
Telmisartan	Poorly soluble	Yes	Failed (2 violation)	0.85
Mizolastine	Moderately soluble	Yes	Passed (0 violation)	0.55

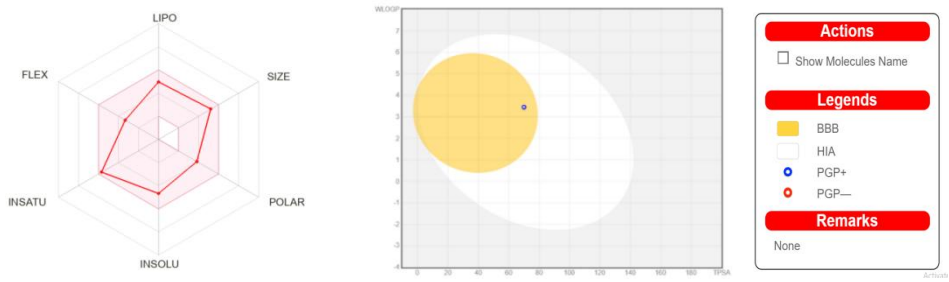


Fig. 3.4 Bioavailability radar diagrams and BOILED-EGG images of mizolastine derived by ADME analysis

3.1.5 Toxicity and Carcinogenicity Analysis Result

The summary of results for selected phytochemical compounds and FDA drugs were presented in Table 3.13 and 3.14.

Table 3.13 Toxicity and carcinogenicity exhibited by phytocompounds

IMPAAT ID	Oral rat acute toxicity LD50 (mol/kg)	Max. tolerated dose human (log mg/kg/day)	Carcinogenicity
IMPHY003277	1.926	-0.446	No
IMPHY010687	1.926	-0.446	No
IMPHY005339	1.402	-0.614	No
IMPHY000630	2.25	-0.272	No
IMPHY001483	3.531	-0.34	Yes

Table 3.14 Toxicity and carcinogenicity exhibited by FDA drugs

FDA Drugs	Oral rat acute toxicity LD50 (mol/kg)	Max. tolerated dose human (log mg/kg/day)	Carcinogenicity
Tirilazad	2.873	-0.599	No
Telmisartan	2.482	-0.407	No
Mizolastine	2.777	0.178	No

3.1.6 Cancer Cell Line Toxicity Analysis Result:

The only activities where Pa exceeds Pi are considered viable for an effective compound. The results were expressed as probabilities of being active (Pa) and inactive (Pi). For all hit compounds, Pa was found to be greater than Pi. The recorded results of hit compounds are detailed in the Table 3.15 and 3.16.

Table 3.15 Cancer cell line toxicity shown by selected phytocompounds

IMPPAT ID	Cancer Cell Line	Disease description	Tissue/Organ	Tumor type	Pa>Pi
IMPHY003277	C6	Glioma	Brain	Glioma	0.425 > 0.060
	XF498	Glioma	Brain	Glioma	0.095 > 0.034
IMPHY010687	C6	Glioma	Brain	Glioma	0.436 > 0.010
	SF-539	Glioblastoma	Brain	Glioblastoma	0.198 > 0.144
	U-251	Astrocytoma	Brain	Astrocytoma	0.870 > 0.004
IMPHY005339	SF-295	Glioblastoma	Brain	Glioblastoma	0.555 > 0.018
	SF-539	Glioblastoma	Brain	Glioblastoma	0.340 > 0.047
	SF-268	Glioblastoma	Brain	Glioblastoma	0.296 > 0.075
	SNB-75	Glioblastoma	Nervous system	Glioblastoma	0.204 > 0.175
	Hs683	Glioma	Brain	Glioma	0.073 > 0.052
IMPHY000630	SNB-19	Astrocytoma	Brain	Astrocytoma	0.175 > 0.048
	C6	Glioma	Brain	Glioma	0.164 > 0.094
IMPHY001483	SF-295	Glioblastoma	Brain	Glioblastoma	0.562 > 0.017
	SF-539	Glioblastoma	Brain	Glioblastoma	0.354 > 0.042
	A172	Glioblastoma	Brain	Glioblastoma	0.351 > 0.108
	SF-268	Glioblastoma	Brain	Glioblastoma	0.311 > 0.068
	U-251	Astrocytoma	Brain	Astrocytoma	0.220 > 0.150
	SNB-19	Astrocytoma	Brain	Astrocytoma	0.141 > 0.079
	Hs683	Glioma	Brain	Glioma	0.077 > 0.042

Table 3.16 Cancer cell line toxicity shown by selected FDA approved drugs

FDA Drugs	Cancer Cell Line	Disease description	Tissue/ Organ	Tumor type	Pa>Pi
Tirilazad	SNB-19	Astrocytoma	Brain	Astrocytoma	0.120 > 0.116
Telmisartan	XF498	Glioma	Brain	Glioma	0.081 > 0.063
Mizolastine	Hs 683	Oligodendroglioma	Brain	Glioma	0.295 > 0.224

3.1.7 Docking Results with STAT1

Among the five selected phytocompounds, IMPHY010687 exhibited the highest binding affinity of -9.1 kcal/mol with STAT1. It was followed by IMPHY000630 and IMPHY003277. The remaining two compounds showed lower docking scores with STAT1. Comparative docking results of these compounds with STAT1 and STAT3 are summarized in the Table 3.17.

Among the FDA-approved drugs listed in the Table 3.18, mizolastine shows the highest activity with a value of -9.5 kcal/mol, outperforming both tirilazad and telmisartan.

Table 3.17 Comparison of docking scores for STAT1 with selected phytocompounds

Phytochemicals		
IMPAAT ID	**STAT3**	**STAT1**
IMPHY003277	-8.9	-8.7
IMPHY010687	-8.8	-9.1
IMPHY005339	-8.7	-7.6
IMPHY000630	-8.6	-8.8
IMPHY001483	-8.5	-7.1

Table 3.18 Comparison of docking scores for STAT1 with selected FDA drugs

FDA Approved Drugs		
Compounds	**STAT3**	**STAT1**
Tirilazad	-9.5	-8.9
Telmisartan	-9.1	-8.1
Mizolastine	-9	-9.5

3.2. DISCUSSION

STAT3 is an oncogenic signal transducer involved in regulates the expression of various genes which significantly shows association with various tumor hallmarks as well as glioblastoma. This study targets this STAT3 and involves two investigations: one focusing on phytocompounds and the other on FDA-approved drugs.

For the first study, Curcumin, a renowned anticancer compound found in the Curcuma longa plant, was used as a reference drug for identifying potential lead compounds. Curcumin is known to regulate the JAK/STAT pathway by reducing or inhibiting STAT3 phosphorylation and preventing its translocation to the nucleus in various cancer cells [166], [167]. Additionally, it has been shown to suppress the activity of STAT1, JAK1, and JAK2 in microglial cells [168]. The reference compound achieved binding score of 6.5 kcal/mol and a validation docking score of 7.3 kcal/mol using CB-Dock2. A docking threshold of -8.1 kcal/mol or lower, was established to identify natural STAT3 inhibitors.

Table 3.1 shows the phytochemicals within binding affinity scores within the threshold range. Out of them top 5 phytocompounds, withametelin or daturilin and ID IMPHY003277 has most negative binding energy. Withametelin (IMPHY003277) and isowithametelin (IMPHY010687) are the phytocompounds derived from the leaf of Datura metel plant belonging to class of steroids based on chemical classification. Withametelin was found to possess antifungal and neuroprotective activity along with cytotoxic effect against various cancer cell lines [169], [170], [171]. Similarly, isowithametelin also have cytotoxic activity and cancer chemopreventive potential [172]. Anolobine (IMPHY005339) is an alkaloid derived from annona squamosa and magnolia grandiflora possess antimicrobial properties. Other compounds investigated included Withasomidienone (IMPHY000630), a steroid isolated from the roots of Withania somnifera, and Xylopine (IMPHY001483), another alkaloid derived from Annona squamosa.

In another study involving FDA-approved drugs, napabucasin was used as a reference. This small molecule is known to reduce the expression of STAT3 and genes related to stemness, and it plays specific roles in regulating the cell cycle, proliferation, invasion and apoptosis [173].

FDA-approved drugs retrieved from DrugBank, along with a reference drug, were subjected to docking. Napabucasin achieved a docking score of -7.5 kcal/mol. A threshold of ≤ -8.5 kcal/mol was set for selecting inhibitor drugs. Those meeting the threshold underwent BBB analysis, resulting in 10 compounds. The top three - tirilazad, telmisartan, and mizolastine which were selected for further analysis.
Further docking validation using CB-Dock2 confirmed these compounds as effective inhibitors of the target protein.

The physicochemical properties of lead compounds are critical for assessing drug-likeness. Key among these properties is the molecular weight, which should ideally be ≤ 500, adhering to lipinski's rule of five, that also limits hydrogen bond donors to 5 and acceptors to 10 [174]. Lipophilicity, measured by the partition coefficient, should be less than 5. Another crucial factor influencing a molecule's bioavailability is its topological polar surface area (TPSA), which should ideally be less than 140 $Å^2$ to enhance oral bioavailability. For this study, the consensus Log Po/w value, calculated as the average of predictions from five different models in Swiss ADME, was used to evaluate lipophilicity. Given these considerations, the lead compounds exhibit excellent physicochemical properties.

The study of drug likeliness and pharmacokinetic properties of the selected compounds underscores that all lead compounds exhibit moderate water solubility and high gastrointestinal absorption while adhering to Lipinski's rule with minimal violations. Additionally, it underscores the essential need for all drugs to cross the

blood-brain barrier, as failing to do so greatly impedes the treatment of neural disorders [175]. The enzymes CYP1A2, CYP2C19, CYP2C9, CYP2D6, and CYP3A4, members of the Cytochrome P450 family, are pivotal in metabolizing numerous drugs. Inhibition of these enzymes can result in significant drug toxicity, underscoring their importance in the pharmacokinetics [176]. Except xlyopine (IMPHY001483), all the compounds were non-inhibitor of these enzymes.

The Table 3.10, 3.11 and 3.12 illustrates the pharmacological profiles of leading FDA-approved drugs. Mizolastine exhibits better physicochemical properties than tirilazad and telmisartan, both of which possess high molecular weights, with tirilazad also having lipophilicity beyond the threshold. In terms of drug-likeness and pharmacokinetics, tirilazad and mizolastine conform to lipinski's rule of five, with a bioavailability score of 0.55. Tirilazad and telmisartan are effective cytochrome P450 enzyme inhibitors but exhibit poor solubility, whereas mizolastine shows moderate solubility.

PkCSM was used for predicting oral rat acute toxicity LD50 and max. tolerated dose in human. The carcinogenicity assessment was performed using CarcinoPred-EL. These analysis indicates phytocompound xlyopine (IMPHY001483) is unsuitable compound due to its carcinogenicity. The toxicity assessment of the chosen compounds unveils their respective toxicity profiles. Notably, the analysis indicates that among the FDA-approved drugs, telmisartan, tirilazad, and mizolastine exhibit non-carcinogenic properties.

The results of the cancer cell line toxicity analysis were positive, as each phytocompound and the selected drug exhibited a Pa value higher than its Pi value. Specifically, compounds anolobine (IMPHY005339) and xylopine (IMPHY001483) demonstrated toxicity to various glioblastoma cancer cell lines.

In both studies, we explored the potential of phytocompounds and FDA drugs, known to be potent STAT3 inhibitors, against STAT1, given that both STAT3 and STAT1 are upregulated in GBM and play significant roles in JAK/STAT pathway and various cancers. We found that all the FDA approved drugs and three of phytocompounds exhibited strong binding affinity with STAT1, yielding promising results.

CHAPTER - 4

CONCLUSION, FUTURE PROSPECTS AND SOCIAL IMPACT

Glioblastoma, the deadliest of all gliomas attracts significant global interest despite its relatively low incidence rate due to its heterogeneous nature and high recurrence. Extensive research is underway to develop more effective and non-resistant therapies to control and mitigate GBM progression. To contribute to these efforts, two synergistic studies were conducted using an in-silico approach to identify effective targets and discover novel inhibitors. The first study focused on natural inhibitors, while the second utilized FDA-approved drugs. Both approaches aimed to target the identified protein more efficiently, ensuring greater safety and fewer side effects compared to other inhibitors.

This method utilizes molecular docking, ADME analysis, toxicity, carcinogenicity and cancer cell line toxicity studies to investigate the potential of phytochemicals from Indian medicinal plants and currently known FDA-approved drugs. The results indicate that both the phytocompounds and FDA-approved drugs effectively inhibit STAT3 and STAT1, crucial signal transducers in the JAK-STAT signaling pathway, which are significant in the context of Glioblastoma.

Based on molecular docking studies, the phytocompounds withametelin (IMPHY003277), isowithametelin (IMPHY010687), anolobine (IMPHY005339), withasomidienone (IMPHY000630) and xylopine (IMPHY001483) have been identified as potent inhibitors. For FDA-approved drugs, tirilazad, telmisartan, and mizolastine were identified as effective candidates with superior docking scores compared to selected phytocompounds. The ability of these compounds to cross BBB could enhance their therapeutic effectiveness. Comprehensive analysis of physicochemical properties, drug-likeness, and pharmacokinetics revealed that phytocompounds generally performed better than FDA-approved drugs, with only the phytocompound xylopine (IMPHY001483) acting as an inhibitor of most drug-metabolizing enzymes. Toxicity and carcinogenicity assessments showed xylopine (IMPHY001483) being carcinogenic along with the relative toxicity profiles of selected compounds. In-silico cancer cell line toxicity tests indicated that all selected candidates were toxic to various brain tissue cell lines, but phytochemicals were overall more effective, showing toxicity to a wider range of brain tissue cell lines. Finally, these potential candidates were docked with STAT1, yielding good docking scores for all except anolobine (IMPHY005339) and xylopine (IMPHY001483). Comparing the results of phytocompounds and FDA drugs, FDA drugs were clear winners based on molecular docking scores. However, in other pharmacological

analysis, the phytocompounds demonstrated better performance. Moreover, further studies are needed for validation and examination of these findings.

Identifying potential targets and inhibitors for glioblastoma holds immense promise for transforming patient care and the broader healthcare landscape. By developing more effective therapies, there is a potential to significantly extend survival rates and enhance the quality of life for glioblastoma patients. This breakthrough could also alleviate the burden on healthcare systems by providing better treatment options, thus optimizing resource allocation and streamlining patient management. Moreover, the prospect of personalized treatment plans tailored to individual patients offers hope and empowerment to those facing this challenging diagnosis. Furthermore, successful identification of these targets and inhibitors has the potential to catalyze increased awareness, research, and investment in glioblastoma treatment, ultimately fostering collaboration and innovation within the scientific community. Overall, this advancement has the capacity to drive profound social impact by improving patient outcomes, reducing healthcare burdens, raising disease awareness and empowering researchers and clinicians in the ongoing fight against glioblastoma.

REFRENCES

[1] L. M. DeAngelis, "Brain Tumors," *New England Journal of Medicine*, vol. 344, no. 2, pp. 114–123, Jan. 2001, doi: 10.1056/NEJM200101113440207.

[2] E. Crocetti *et al.*, "Epidemiology of glial and non-glial brain tumours in Europe," *Eur J Cancer*, vol. 48, no. 10, pp. 1532–1542, Jul. 2012, doi: 10.1016/j.ejca.2011.12.013.

[3] H. Jiang, Y. Cui, J. Wang, and S. Lin, "Impact of epidemiological characteristics of supratentorial gliomas in adults brought about by the 2016 world health organization classification of tumors of the central nervous system," *Oncotarget*, vol. 8, no. 12, pp. 20354–20361, Mar. 2017, doi: 10.18632/oncotarget.13555.

[4] D. N. Louis *et al.*, "The 2007 WHO Classification of Tumours of the Central Nervous System," *Acta Neuropathol*, vol. 114, no. 2, pp. 97–109, Aug. 2007, doi: 10.1007/s00401-007-0243-4.

[5] B. Grobben, P. De Deyn, and H. Slegers, "Rat C6 glioma as experimental model system for the study of glioblastoma growth and invasion," *Cell Tissue Res*, vol. 310, no. 3, pp. 257–270, Dec. 2002, doi: 10.1007/s00441-002-0651-7.

[6] B. Grobben, P. De Deyn, and H. Slegers, "Rat C6 glioma as experimental model system for the study of glioblastoma growth and invasion," *Cell Tissue Res*, vol. 310, no. 3, pp. 257–270, Dec. 2002, doi: 10.1007/s00441-002-0651-7.

[7] E. C. Holland, "Glioblastoma multiforme: The terminator," *Proceedings of the National Academy of Sciences*, vol. 97, no. 12, pp. 6242–6244, Jun. 2000, doi: 10.1073/pnas.97.12.6242.

[8] Q. T. Ostrom *et al.*, "CBTRUS Statistical Report: Primary Brain and Other Central Nervous System Tumors Diagnosed in the United States in 2015–2019," *Neuro Oncol*, vol. 24, no. Supplement_5, pp. v1–v95, Oct. 2022, doi: 10.1093/neuonc/noac202.

[9] R. Stupp *et al.*, "Radiotherapy plus Concomitant and Adjuvant Temozolomide for Glioblastoma," *New England Journal of Medicine*, vol. 352, no. 10, pp. 987–996, Mar. 2005, doi: 10.1056/NEJMoa043330.

[10] R. Siegel, J. Ma, Z. Zou, and A. Jemal, "Cancer statistics, 2014," *CA Cancer J Clin*, vol. 64, no. 1, pp. 9–29, Jan. 2014, doi: 10.3322/caac.21208.

[11] L. C. Hou, A. Veeravagu, A. R. Hsu, and V. C. K. Tse, "Recurrent glioblastoma multiforme: a review of natural history and management options," *Neurosurg Focus*, vol. 20, no. 4, p. E3, Apr. 2006, doi: 10.3171/foc.2006.20.4.2.

[12] A. Ou, M. Ott, D. Fang, and A. Heimberger, "The Role and Therapeutic Targeting of JAK/STAT Signaling in Glioblastoma," *Cancers (Basel)*, vol. 13, no. 3, p. 437, Jan. 2021, doi: 10.3390/cancers13030437.

[13] Y.-J. Zhang *et al.*, "Antioxidant Phytochemicals for the Prevention and Treatment of Chronic Diseases," *Molecules*, vol. 20, no. 12, pp. 21138–21156, Nov. 2015, doi: 10.3390/molecules201219753.

[14] R. Vidya Priyadarsini and S. Nagini, "Cancer Chemoprevention by Dietary Phytochemicals: Promises and Pitfalls," *Curr Pharm Biotechnol*, vol. 13, no. 1, pp. 125–136, Jan. 2012, doi: 10.2174/138920112798868610.

[15] D. N. Louis *et al.*, "The 2016 World Health Organization Classification of Tumors of the Central Nervous System: a summary," *Acta Neuropathol*, vol. 131, no. 6, pp. 803–820, Jun. 2016, doi: 10.1007/s00401-016-1545-1.

[16] R. G. W. Verhaak *et al.*, "Integrated Genomic Analysis Identifies Clinically Relevant Subtypes of Glioblastoma Characterized by Abnormalities in PDGFRA, IDH1, EGFR, and NF1," *Cancer Cell*, vol. 17, no. 1, pp. 98–110, Jan. 2010, doi: 10.1016/j.ccr.2009.12.020.

[17] Mesfin FB and Al-Dhahir MA, *Gliomas*. 2023.

[18] Q. T. Ostrom *et al.*, "CBTRUS Statistical Report: Primary Brain and Other Central Nervous System Tumors Diagnosed in the United States in 2012–2016," *Neuro Oncol*, vol. 21, no. Supplement_5, pp. v1–v100, Nov. 2019, doi: 10.1093/neuonc/noz150.

[19] T. Tykocki and M. Eltayeb, "Ten-year survival in glioblastoma. A systematic review," *Journal of Clinical Neuroscience*, vol. 54, pp. 7–13, Aug. 2018, doi: 10.1016/j.jocn.2018.05.002.

[20] K. Urbańska, J. Sokołowska, M. Szmidt, and P. Sysa, "Review Glioblastoma multiforme – an overview," *Współczesna Onkologia*, vol. 5, pp. 307–312, 2014, doi: 10.5114/wo.2014.40559.

[21] V. Gilard *et al.*, "Diagnosis and Management of Glioblastoma: A Comprehensive Perspective," *J Pers Med*, vol. 11, no. 4, p. 258, Apr. 2021, doi: 10.3390/jpm11040258.

[22] S. Schultz, G. S. Pinsky, N. C. Wu, M. C. Chamberlain, A. S. Rodrigo, and S. E. Martin, "Fine needle aspiration diagnosis of extracranial glioblastoma multiforme: Case report and review of the literature," *Cytojournal*, vol. 2, no. 1, p. 19, 2005, doi: 10.1186/1742-6413-2-19.

[23] K. Urbańska, J. Sokołowska, M. Szmidt, and P. Sysa, "Review Glioblastoma multiforme – an overview," *Współczesna Onkologia*, vol. 5, pp. 307–312, 2014, doi: 10.5114/wo.2014.40559.

[24] A. M. Rojiani and K. Dorovini-Zis, "Glomeruloid vascular structures in glioblastoma multiforme: an immunohistochemical and ultrastructural study," *J Neurosurg*, vol. 85, no. 6, pp. 1078–1084, Dec. 1996, doi: 10.3171/jns.1996.85.6.1078.

[25] L. Rong, N. Li, and Z. Zhang, "Emerging therapies for glioblastoma: current state and future directions," *Journal of Experimental & Clinical Cancer Research*, vol. 41, no. 1, p. 142, Dec. 2022, doi: 10.1186/s13046-022-02349-7.

[26] S. Tiwari and Z. Han, "Immunotherapy: Advancing glioblastoma treatment—A narrative review of scientific studies," *Cancer Rep*, Dec. 2023, doi: 10.1002/cnr2.1947.

[27] E. Agosti *et al.*, "Glioblastoma Immunotherapy: A Systematic Review of the Present Strategies and Prospects for Advancements," *Int J Mol Sci*, vol. 24, no. 20, p. 15037, Oct. 2023, doi: 10.3390/ijms242015037.

[28] A. Olar and K. D. Aldape, "Using the molecular classification of glioblastoma to inform personalized treatment," *J Pathol*, vol. 232, no. 2, pp. 165–177, Jan. 2014, doi: 10.1002/path.4282.

[29] G. S. Stoyanov, D. Dzhenkov, P. Ghenev, B. Iliev, Y. Enchev, and A. B. Tonchev, "Cell biology of glioblastoma multiforme: from basic science to diagnosis and treatment," *Medical Oncology*, vol. 35, no. 3, p. 27, Mar. 2018, doi: 10.1007/s12032-018-1083-x.

[30] S. Kesari, "Understanding Glioblastoma Tumor Biology: The Potential to Improve Current Diagnosis and Treatments," *Semin Oncol*, vol. 38, pp. S2–S10, Dec. 2011, doi: 10.1053/j.seminoncol.2011.09.005.

[31] S. Karcher *et al.*, "Different angiogenic phenotypes in primary and secondary glioblastomas," *Int J Cancer*, vol. 118, no. 9, pp. 2182–2189, May 2006, doi: 10.1002/ijc.21648.

[32] R. Stupp *et al.*, "Radiotherapy plus Concomitant and Adjuvant Temozolomide for Glioblastoma," *New England Journal of Medicine*, vol. 352, no. 10, pp. 987–996, Mar. 2005, doi: 10.1056/NEJMoa043330.

[33] M. Mahvash, H.-H. Hugo, H. Maslehaty, H. M. Mehdorn, and A. M. Stark, "Glioblastoma Multiforme in Children: Report of 13 Cases and Review of the Literature," *Pediatr Neurol*, vol. 45, no. 3, pp. 178–180, Sep. 2011, doi: 10.1016/j.pediatrneurol.2011.05.004.

[34] M. G. Castro *et al.*, "Gene Therapy and Targeted Toxins for Glioma," *Curr Gene Ther*, vol. 11, no. 3, pp. 155–180, Jun. 2011, doi: 10.2174/156652311795684722.

[35 M. C. Chamberlain, "Bevacizumab for the Treatment of Recurrent Glioblastoma," *Clin Med Insights Oncol*, vol. 5, p. CMO.S7232, Jan. 2011, doi: 10.4137/CMO.S7232.

[36] N. ALTIOK, M. ERSOZ, and M. KOYUTURK, "Estradiol induces JNK-dependent apoptosis in glioblastoma cells," *Oncol Lett*, vol. 2, no. 6, pp. 1281–1285, Nov. 2011, doi: 10.3892/ol.2011.385.

[37] S. Tiwari and Z. Han, "Immunotherapy: Advancing glioblastoma treatment—A narrative review of scientific studies," *Cancer Rep*, vol. 7, no. 2, Feb. 2024, doi: 10.1002/cnr2.1947.

[38] J. Wahyuhadi *et al.*, "Active Immunotherapy for Glioblastoma Treatment: A Systematic Review and Meta-Analysis," *Cancer Control*, vol. 29, p. 107327482210794, Sep. 2022, doi: 10.1177/10732748221079474.

[39] X. Hu, J. li, M. Fu, X. Zhao, and W. Wang, "The JAK/STAT signaling pathway: from bench to clinic," *Signal Transduct Target Ther*, vol. 6, no. 1, p. 402, Nov. 2021, doi: 10.1038/s41392-021-00791-1.

[40] J. E. Darnell, "STATs and Gene Regulation," *Science (1979)*, vol. 277, no. 5332, pp. 1630–1635, Sep. 1997, doi: 10.1126/science.277.5332.1630.

[41] K. L. Owen, N. K. Brockwell, and B. S. Parker, "JAK-STAT Signaling: A Double-Edged Sword of Immune Regulation and Cancer Progression," *Cancers (Basel)*, vol. 11, no. 12, p. 2002, Dec. 2019, doi: 10.3390/cancers11122002.

[42] J. Bromberg and J. E. Darnell, "The role of STATs in transcriptional control and their impact on cellular function," *Oncogene*, vol. 19, no. 21, pp. 2468–2473, May 2000, doi: 10.1038/sj.onc.1203476.

[43] J. F. Bromberg *et al.*, "Stat3 as an Oncogene," *Cell*, vol. 98, no. 3, pp. 295–303, Aug. 1999, doi: 10.1016/S0092-8674(00)81959-5.

[44] R. M. Ransohoff, "How neuroinflammation contributes to neurodegeneration," *Science (1979)*, vol. 353, no. 6301, pp. 777–783, Aug. 2016, doi: 10.1126/science.aag2590.

[45] P. C. Tiwari and R. Pal, "The potential role of neuroinflammation and transcription factors in Parkinson disease," *Dialogues Clin Neurosci*, vol. 19, no. 1, pp. 71–80, Mar. 2017, doi: 10.31887/DCNS.2017.19.1/rpal.

[46] X. Hu, J. li, M. Fu, X. Zhao, and W. Wang, "The JAK/STAT signaling pathway: from bench to clinic," *Signal Transduct Target Ther*, vol. 6, no. 1, p. 402, Nov. 2021, doi: 10.1038/s41392-021-00791-1.

[47] D. W. Dodington, H. R. Desai, and M. Woo, "JAK/STAT – Emerging Players in Metabolism," *Trends in Endocrinology & Metabolism*, vol. 29, no. 1, pp. 55–65, Jan. 2018, doi: 10.1016/j.tem.2017.11.001.

[48] E. Bousoik and H. Montazeri Aliabadi, "'Do We Know Jack' About JAK? A Closer Look at JAK/STAT Signaling Pathway," *Front Oncol*, vol. 8, Jul. 2018, doi: 10.3389/fonc.2018.00287.

[49] P. J. Lupardus, M. Ultsch, H. Wallweber, P. Bir Kohli, A. R. Johnson, and C. Eigenbrot, "Structure of the pseudokinase–kinase domains from protein kinase TYK2 reveals a mechanism for Janus kinase (JAK) autoinhibition," *Proceedings of the National Academy of Sciences*, vol. 111, no. 22, pp. 8025–8030, Jun. 2014, doi: 10.1073/pnas.1401180111.

[50] H. Yu and R. Jove, "The STATs of cancer — new molecular targets come of age," *Nat Rev Cancer*, vol. 4, no. 2, pp. 97–105, Feb. 2004, doi: 10.1038/nrc1275.

[51] T. Decker and P. Kovarik, "Serine phosphorylation of STATs," *Oncogene*, vol. 19, no. 21, pp. 2628–2637, May 2000, doi: 10.1038/sj.onc.1203481.

[52] I. Strehlow and C. Schindler, "Amino-terminal Signal Transducer and Activator of Transcription (STAT) Domains Regulate Nuclear Translocation and STAT Deactivation," *Journal of Biological Chemistry*, vol. 273, no. 43, pp. 28049–28056, Oct. 1998, doi: 10.1074/jbc.273.43.28049.

[53] U. Vinkemeier, I. Moarefi, J. E. Darnell, and J. Kuriyan, "Structure of the Amino-Terminal Protein Interaction Domain of STAT-4," *Science (1979)*, vol. 279, no. 5353, pp. 1048–1052, Feb. 1998, doi: 10.1126/science.279.5353.1048.

[54] C. P. Lim and X. Cao, “Structure, function, and regulation of STAT proteins,” *Mol Biosyst*, vol. 2, no. 11, p. 536, 2006, doi: 10.1039/b606246f.

[55] M. Zhu, S. John, M. Berg, and W. J. Leonard, “Functional Association of Nmi with Stat5 and Stat1 in IL-2- and IFN γ-Mediated Signaling,” *Cell*, vol. 96, no. 1, pp. 121–130, Jan. 1999, doi: 10.1016/S0092-8674(00)80965-4.

[56] S. Brown, N. Hu, and J. C.-G. Hombría, “Novel level of signalling control in the JAK/STAT pathway revealed by in situ visualisation of protein-protein interaction during *Drosophila* development,” *Development*, vol. 130, no. 14, pp. 3077–3084, Jul. 2003, doi: 10.1242/dev.00535.

[57] T. Kawata *et al.*, “SH2 Signaling in a Lower Eukaryote: A STAT Protein That Regulates Stalk Cell Differentiation in Dictyostelium,” *Cell*, vol. 89, no. 6, pp. 909–916, Jun. 1997, doi: 10.1016/S0092-8674(00)80276-7.

[58] T. Kisseleva, S. Bhattacharya, J. Braunstein, and C. W. Schindler, “Signaling through the JAK/STAT pathway, recent advances and future challenges,” *Gene*, vol. 285, no. 1–2, pp. 1–24, Feb. 2002, doi: 10.1016/S0378-1119(02)00398-0.

[59] K. D. Liu, S. L. Gaffen, and M. A. Goldsmith, “JAK/STAT signaling by cytokine receptors,” *Curr Opin Immunol*, vol. 10, no. 3, pp. 271–278, Jun. 1998, doi: 10.1016/S0952-7915(98)80165-9.

[60] T. Decker and P. Kovarik, “Serine phosphorylation of STATs,” *Oncogene*, vol. 19, no. 21, pp. 2628–2637, May 2000, doi: 10.1038/sj.onc.1203481.

[61] Q. Gao, X. Liang, A. S. Shaikh, J. Zang, W. Xu, and Y. Zhang, “JAK/STAT Signal Transduction: Promising Attractive Targets for Immune, Inflammatory and Hematopoietic Diseases,” *Curr Drug Targets*, vol. 19, no. 5, pp. 487–500, Mar. 2018, doi: 10.2174/1389450117666161207163054.

[62] S. Aittomäki and M. Pesu, “Therapeutic Targeting of the <scp>JAK</scp> / <scp>STAT</scp> Pathway,” *Basic Clin Pharmacol Toxicol*, vol. 114, no. 1, pp. 18–23, Jan. 2014, doi: 10.1111/bcpt.12164.

[63] Ö. Gündüz, “JAK/STAT pathway modulation: Does it work in dermatology?,” *Dermatol Ther*, vol. 32, no. 3, May 2019, doi: 10.1111/dth.12903.

[64] P. J. Murray, “The JAK-STAT Signaling Pathway: Input and Output Integration,” *The Journal of Immunology*, vol. 178, no. 5, pp. 2623–2629, Mar. 2007, doi: 10.4049/jimmunol.178.5.2623.

[65] K. Swiatek-Machado and B. Kaminska, “STAT Signaling in Glioma Cells,” 2013, pp. 189–208. doi: 10.1007/978-94-007-4719-7_10.

[66] F. Shao, X. Pang, and G. H. Baeg, “Targeting the JAK/STAT Signaling Pathway for Breast Cancer,” *Curr Med Chem*, vol. 28, no. 25, pp. 5137–5151, Aug. 2021, doi: 10.2174/0929867328666201207202012.

[67] M. Zhang *et al.*, “A STAT3 palmitoylation cycle promotes TH17 differentiation and colitis,” *Nature*, vol. 586, no. 7829, pp. 434–439, Oct. 2020, doi: 10.1038/s41586-020-2799-2.

[68] L. Yang *et al.*, “Targeting cancer stem cell pathways for cancer therapy,” *Signal Transduct Target Ther*, vol. 5, no. 1, p. 8, Feb. 2020, doi: 10.1038/s41392-020-0110-5.

[69] X. Hu, J. li, M. Fu, X. Zhao, and W. Wang, “The JAK/STAT signaling pathway: from bench to clinic,” *Signal Transduct Target Ther*, vol. 6, no. 1, p. 402, Nov. 2021, doi: 10.1038/s41392-021-00791-1.

[70] H. Yu, H. Lee, A. Herrmann, R. Buettner, and R. Jove, “Revisiting STAT3 signalling in cancer: new and unexpected biological functions,” *Nat Rev Cancer*, vol. 14, no. 11, pp. 736–746, Nov. 2014, doi: 10.1038/nrc3818.

[71] M. Priester *et al.*, “STAT3 silencing inhibits glioma single cell infiltration and tumor growth,” *Neuro Oncol*, vol. 15, no. 7, pp. 840–852, Jul. 2013, doi: 10.1093/neuonc/not025.

[72] C. Lindemann, O. Hackmann, S. Delic, N. Schmidt, G. Reifenberger, and M. J. Riemenschneider, “SOCS3 promoter methylation is mutually exclusive to EGFR

amplification in gliomas and promotes glioma cell invasion through STAT3 and FAK activation," *Acta Neuropathol*, vol. 122, no. 2, pp. 241–251, Aug. 2011, doi: 10.1007/s00401-011-0832-0.

[73] G. Zadeh, K. P. L. Bhat, and K. Aldape, "EGFR and EGFRvIII in Glioblastoma: Partners in Crime," *Cancer Cell*, vol. 24, no. 4, pp. 403–404, Oct. 2013, doi: 10.1016/j.ccr.2013.09.017.

[74] O. A. Guryanova *et al.*, "Nonreceptor Tyrosine Kinase BMX Maintains Self-Renewal and Tumorigenic Potential of Glioblastoma Stem Cells by Activating STAT3," *Cancer Cell*, vol. 19, no. 4, pp. 498–511, Apr. 2011, doi: 10.1016/j.ccr.2011.03.004.

[75] Q. Xu *et al.*, "Targeting Stat3 blocks both HIF-1 and VEGF expression induced by multiple oncogenic growth signaling pathways," *Oncogene*, vol. 24, no. 36, pp. 5552–5560, Aug. 2005, doi: 10.1038/sj.onc.1208719.

[76] H. Park *et al.*, "Rubiarbonone C inhibits platelet-derived growth factor-induced proliferation and migration of vascular smooth muscle cells through the focal adhesion kinase, MAPK and STAT3 Tyr ^{705} signalling pathways," *Br J Pharmacol*, vol. 174, no. 22, pp. 4140–4154, Nov. 2017, doi: 10.1111/bph.13986.

[77] H. Yu, S. Zhang, A. N. Ibrahim, J. Wang, Z. Deng, and M. Wang, "RCC2 promotes proliferation and radio-resistance in glioblastoma via activating transcription of DNMT1," *Biochem Biophys Res Commun*, vol. 516, no. 3, pp. 999–1006, Aug. 2019, doi: 10.1016/j.bbrc.2019.06.097.

[78] D. Kesanakurti, C. Chetty, D. Rajasekhar Maddirela, M. Gujrati, and J. S. Rao, "Essential role of cooperative NF-κB and Stat3 recruitment to ICAM-1 intronic consensus elements in the regulation of radiation-induced invasion and migration in glioma," *Oncogene*, vol. 32, no. 43, pp. 5144–5155, Oct. 2013, doi: 10.1038/onc.2012.546.

[79] W. Fu, X. Hou, L. Dong, and W. Hou, "Roles of STAT3 in the pathogenesis and treatment of glioblastoma," *Front Cell Dev Biol*, vol. 11, Feb. 2023, doi: 10.3389/fcell.2023.1098482.

[80] A. Jhaveri, P. Deshpande, B. Pattni, and V. Torchilin, "Transferrin-targeted, resveratrol-loaded liposomes for the treatment of glioblastoma," *Journal of Controlled Release*, vol. 277, pp. 89–101, May 2018, doi: 10.1016/j.jconrel.2018.03.006.

[81] M. Ott *et al.*, "Radiation with STAT3 Blockade Triggers Dendritic Cell–T cell Interactions in the Glioma Microenvironment and Therapeutic Efficacy," *Clinical Cancer Research*, vol. 26, no. 18, pp. 4983–4994, Sep. 2020, doi: 10.1158/1078-0432.CCR-19-4092.

[82] T. C. Dale, A. M. Imam, I. M. Kerr, and G. R. Stark, "Rapid activation by interferon alpha of a latent DNA-binding protein present in the cytoplasm of untreated cells.," *Proceedings of the National Academy of Sciences*, vol. 86, no. 4, pp. 1203–1207, Feb. 1989, doi: 10.1073/pnas.86.4.1203.

[83] D. E. Levy, D. S. Kessler, R. Pine, and J. E. Darnell, "Cytoplasmic activation of ISGF3, the positive regulator of interferon-alpha-stimulated transcription, reconstituted in vitro.," *Genes Dev*, vol. 3, no. 9, pp. 1362–1371, Sep. 1989, doi: 10.1101/gad.3.9.1362.

[84] K. Yamamoto, H. Kobayashi, A. Arai, O. Miura, S. Hirosawa, and N. Miyasaka, "cDNA cloning, expression and chromosome mapping of the human STAT4 gene: both STAT4 and STAT1 genes are mapped to 2q32.2→q32.3," *Cytogenet Genome Res*, vol. 77, no. 3–4, pp. 207–210, 1997, doi: 10.1159/000134578.

[85] L. Zhao, X. Li, J. Su, F. Wang Gong, J. Lu, and Y. Wei, "*STAT1* determines aggressiveness of glioblastoma both in vivo and in vitro through wnt/β-catenin signalling pathway," *Cell Biochem Funct*, vol. 38, no. 5, pp. 630–641, Jul. 2020, doi: 10.1002/cbf.3518.

[86] M. Hiroi, K. Mori, Y. Sakaeda, J. Shimada, and Y. Ohmori, "STAT1 represses hypoxia-inducible factor-1-mediated transcription," *Biochem Biophys Res Commun*, vol. 387, no. 4, pp. 806–810, Oct. 2009, doi: 10.1016/j.bbrc.2009.07.138.

[87] H. Ju *et al.*, "Mediation of multiple pathways regulating cell proliferation, migration, and apoptosis in the human malignant glioma cell line U87MG via unphosphorylated STAT1," *J Neurosurg*, vol. 118, no. 6, pp. 1239–1247, Jun. 2013, doi: 10.3171/2013.3.JNS122051.

[88] S. X. Zamora-Salas, M. Macías-Silva, and A. C. Tecalco-Cruz, "Upregulation of the canonical signaling pathway of interferon-gamma is associated with glioblastoma progression," *Mol Biol Rep*, vol. 51, no. 1, p. 64, Dec. 2024, doi: 10.1007/s11033-023-09062-4.

[89] C. W. Duarte *et al.*, "Expression Signature of IFN/STAT1 Signaling Genes Predicts Poor Survival Outcome in Glioblastoma Multiforme in a Subtype-Specific Manner," *PLoS One*, vol. 7, no. 1, p. e29653, Jan. 2012, doi: 10.1371/journal.pone.0029653.

[90] B. Thota *et al.*, "STAT-1 expression is regulated by IGFBP-3 in malignant glioma cells and is a strong predictor of poor survival in patients with glioblastoma," *J Neurosurg*, vol. 121, no. 2, pp. 374–383, Aug. 2014, doi: 10.3171/2014.4.JNS131198.

[91] L. Zhao, X. Li, J. Su, F. Wang Gong, J. Lu, and Y. Wei, "*STAT1* determines aggressiveness of glioblastoma both in vivo and in vitro through wnt/β-catenin signalling pathway," *Cell Biochem Funct*, vol. 38, no. 5, pp. 630–641, Jul. 2020, doi: 10.1002/cbf.3518.

[92] U. Saleem, Z. Raza, F. Anwar, Z. Chaudary, and B. Ahmad, "Systems pharmacology based approach to investigate the in-vivo therapeutic efficacy of Albizia lebbeck (L.) in experimental model of Parkinson's disease," *BMC Complement Altern Med*, vol. 19, no. 1, p. 352, Dec. 2019, doi: 10.1186/s12906-019-2772-5.

[93] P. Venkatesh *et al.*, "Anti-allergic activity of standardized extract of *Albizia lebbeck* with reference to catechin as a phytomarker," *Immunopharmacol Immunotoxicol*, vol. 32, no. 2, pp. 272–276, Jun. 2010, doi: 10.3109/08923970903305481.

[94] S. Kalia, N. Walter, and U. Bagai, "Antimalarial efficacy of Albizia lebbeck (Leguminosae) against Plasmodium falciparum in vitro & P. berghei in vivo," *Indian Journal of Medical Research*, vol. 142, no. 7, p. 101, 2015, doi: 10.4103/0971-5916.176635.

[95] O. N. Avoseh, F. M. Mtunzi, I. A. Ogunwande, R. Ascrizzi, and F. Guido, "Albizia lebbeck and Albizia zygia volatile oils exhibit anti-nociceptive and anti-inflammatory properties in pain models," *J Ethnopharmacol*, vol. 268, p. 113676, Mar. 2021, doi: 10.1016/j.jep.2020.113676.

[96] T. H. Desai and S. V. Joshi, "Anticancer activity of saponin isolated from Albizia lebbeck using various in vitro models," *J Ethnopharmacol*, vol. 231, pp. 494–502, Mar. 2019, doi: 10.1016/j.jep.2018.11.004.

[97] S.Gajalakshmi, R. Divya, V. D. Deepika, and S. Mythili, "Pharmacological activities of Annona squamosa: A review".

[98] A. Kumar, S. Shashni, P. Kumar, D. Pant, A. Singh, and R. K. Verma, "Phytochemical constituents, distributions and traditional usages of Arnebia euchroma: A review," *J Ethnopharmacol*, vol. 271, p. 113896, May 2021, doi: 10.1016/j.jep.2021.113896.

[99] Z.-P. Zhang *et al.*, "Euchronin A–F isolated from the Arnebia euchroma (Royle) Johnst. and their anti-proliferative activities in vitro," *J Nat Med*, vol. 78, no. 1, pp. 33–41, Jan. 2024, doi: 10.1007/s11418-023-01738-2.

[100] N. Kretschmer, C. Durchschein, G. Heubl, E.-M. Pferschy-Wenzig, O. Kunert, and R. Bauer, "Discrimination of Zicao Samples Based on DNA Barcoding and HPTLC Fingerprints, and Identification of (22E)-Ergosta-4,6,8(14),22-tetraen-3-one As a Marker Compound," *Planta Med*, vol. 89, no. 08, pp. 824–832, Jul. 2023, doi: 10.1055/a-1855-1778.

[101] M. SHIMOYAMADA, M. SUZUKI, H. SONTA, M. MARUYAMA, and K. OKUBO, "Antifungal activity of the saponin fraction obtained from Asparagus officinalis L. and its active principle.," *Agric Biol Chem*, vol. 54, no. 10, pp. 2553–2557, 1990, doi: 10.1271/bbb1961.54.2553.

[102] Y. Li *et al.*, "Mechanism of action of Asparagus officinalis extract against multiple myeloma using bioinformatics tools, in silico and in vitro study," *Front Pharmacol*, vol. 14, May 2023, doi: 10.3389/fphar.2023.1076815.

[103] R. Md. Hafizur, N. Kabir, and S. Chishti, "*Asparagus officinalis* extract controls blood glucose by improving insulin secretion and β-cell function in streptozotocin-induced type 2

diabetic rats," *British Journal of Nutrition*, vol. 108, no. 9, pp. 1586–1595, Nov. 2012, doi: 10.1017/S0007114511007148.

[104] S. Alok, S. K. Jain, A. Verma, M. Kumar, A. Mahor, and M. Sabharwal, "Plant profile, phytochemistry and pharmacology of Asparagus racemosus (Shatavari): A review," *Asian Pac J Trop Dis*, vol. 3, no. 3, pp. 242–251, Apr. 2013, doi: 10.1016/S2222-1808(13)60049-3.

[105] K. Sairam, S. Priyambada, N. C. Aryya, and R. K. Goel, "Gastroduodenal ulcer protective activity of Asparagus racemosus: an experimental, biochemical and histological study," *J Ethnopharmacol*, vol. 86, no. 1, pp. 1–10, May 2003, doi: 10.1016/S0378-8741(02)00342-2.

[106] M. S. Ali, I. Azhar, Z. Amtul, V. U. Ahmad, and K. Usmanghani, "Antimicrobial screening of some Caesalpiniaceae," *Fitoterapia*, vol. 70, no. 3, pp. 299–304, Jun. 1999, doi: 10.1016/S0367-326X(99)00015-5.

[107] S. A. Nirmal, V. V. Dhasade, R. B. Laware, R. A. Rathi, and B. S. Kuchekar, "Antihistaminic Effect of Bauhinia racemosa Leaves," *Journal of Young Pharmacists*, vol. 3, no. 2, pp. 129–131, Apr. 2011, doi: 10.4103/0975-1483.80301.

[108] M. Gupta *et al.*, "Anti-inflammatory, analgesic and antipyretic effects of methanol extract from Bauhinia racemosa stem bark in animal models," *J Ethnopharmacol*, vol. 98, no. 3, pp. 267–273, Apr. 2005, doi: 10.1016/j.jep.2005.01.018.

[109] M. S. Ali, I. Azhar, Z. Amtul, V. U. Ahmad, and K. Usmanghani, "Antimicrobial screening of some Caesalpiniaceae," *Fitoterapia*, vol. 70, no. 3, pp. 299–304, Jun. 1999, doi: 10.1016/S0367-326X(99)00015-5.

[110] Y.-M. Chiang, D.-Y. Chuang, S.-Y. Wang, Y.-H. Kuo, P.-W. Tsai, and L.-F. Shyur, "Metabolite profiling and chemopreventive bioactivity of plant extracts from Bidens pilosa," *J Ethnopharmacol*, vol. 95, no. 2–3, pp. 409–419, Dec. 2004, doi: 10.1016/j.jep.2004.08.010.

[111] M. Habeck, "Diabetes treatments get sweet help from nature," *Nat Med*, vol. 9, no. 10, pp. 1228–1228, Oct. 2003, doi: 10.1038/nm1003-1228a.

[112] N. YOSHIDA, T. KANEKURA, Y. HIGASHI, and T. KANZAKI, "*Bidens pilosa* suppresses interleukin-1β-induced cyclooxygenase-2 expression through the inhibition of mitogen activated protein kinases phosphorylation in normal human dermal fibroblasts," *J Dermatol*, vol. 33, no. 10, pp. 676–683, Oct. 2006, doi: 10.1111/j.1346-8138.2006.00158.x.

[113] S. W. Wright *et al.*, "Synthesis, chemical, and biological properties of vinylogous hydroxamic acids: dual inhibitors of 5-lipoxygenase and IL-1 biosynthesis," *J Med Chem*, vol. 35, no. 22, pp. 4061–4068, Oct. 1992, doi: 10.1021/jm00100a011.

[114] P. Karia, K. V. Patel, and S. S. P. Rathod, "Breast cancer amelioration by Butea monosperma in-vitro and in-vivo," *J Ethnopharmacol*, vol. 217, pp. 54–62, May 2018, doi: 10.1016/j.jep.2017.12.026.

[115] P. M. Mazumder, M. K. Das, and S. Das, "Butea Monosperma (LAM.) Kuntze – A Comprehensive Review," *International Journal of Pharmaceutical Sciences and Nanotechnology*, vol. 4, no. 2, pp. 1390–1393, Aug. 2011, doi: 10.37285/ijpsn.2011.4.2.2.

[116] S. Sivapalan, S. Dharmalingam, V. Venkatesan, M. Angappan, and V. Ashokkumar, "Phytochemical analysis, anti-inflammatory, antioxidant activity of Calotropis gigantea and its therapeutic applications," *J Ethnopharmacol*, vol. 303, p. 115963, Mar. 2023, doi: 10.1016/j.jep.2022.115963.

[117] T. Choedon, "Anticancer and cytotoxic properties of the latex of Calotropis procera in a transgenic mouse model of hepatocellular carcinoma," *World J Gastroenterol*, vol. 12, no. 16, p. 2517, 2006, doi: 10.3748/wjg.v12.i16.2517.

[118] M.-H. Huang *et al.*, "Antioxidant and anti-inflammatory properties of Cardiospermum halicacabum and its reference compounds ex vivo and in vivo," *J Ethnopharmacol*, vol. 133, no. 2, pp. 743–750, Jan. 2011, doi: 10.1016/j.jep.2010.11.005.

[119] M. S. Sheeba and V. V. Asha, "Cardiospermum halicacabum ethanol extract inhibits LPS induced COX-2, TNF-α and iNOS expression, which is mediated by NF-κB regulation, in

RAW264.7 cells," *J Ethnopharmacol*, vol. 124, no. 1, pp. 39–44, Jul. 2009, doi: 10.1016/j.jep.2009.04.020.

[120] I. Thabrew, J. Munasinghe, S. Chackrewarthi, and S. Senarath, "The effects of Cassia auriculata and Cardiospermum halicacabum teas on the steady state blood level and toxicity of carbamazepine," *J Ethnopharmacol*, vol. 90, no. 1, pp. 145–150, Jan. 2004, doi: 10.1016/j.jep.2003.09.040.

[121] A. Kumar, V. Singh, and A. K. Chaudhary, "Gastric antisecretory and antiulcer activities of Cedrus deodara (Roxb.) Loud. in Wistar rats," *J Ethnopharmacol*, vol. 134, no. 2, pp. 294–297, Mar. 2011, doi: 10.1016/j.jep.2010.12.019.

[122] M. George and L. Joseph, "Anti-allergic, anti-pruritic, and anti-inflammatory activities of <i>Centella asiatica</i> extracts," *African Journal of Traditional, Complementary and Alternative Medicines*, vol. 6, no. 4, Jul. 2010, doi: 10.4314/ajtcam.v6i4.57206.

[123] J. H. Sampson, A. Raman, G. Karlsen, H. Navsaria, and I. M. Leigh, "In vitro keratinocyte antiproliferant effect of Centella asiatica extract and triterpenoid saponins," *Phytomedicine*, vol. 8, no. 3, pp. 230–235, Jan. 2001, doi: 10.1078/0944-7113-00032.

[124] C. L. Cheng, J. S. Guo, J. Luk, and M. W. L. Koo, "The healing effects of Centella extract and asiaticoside on acetic acid induced gastric ulcers in rats," *Life Sci*, vol. 74, no. 18, pp. 2237–2249, Mar. 2004, doi: 10.1016/j.lfs.2003.09.055.

[125] X.-S. Wang, Q. Dong, J.-P. Zuo, and J.-N. Fang, "Structure and potential immunological activity of a pectin from Centellaasiatica (L.) Urban," *Carbohydr Res*, vol. 338, no. 22, pp. 2393–2402, Oct. 2003, doi: 10.1016/S0008-6215(03)00380-X.

[126] A. Gnanapragasam, K. Kumar Ebenezar, V. Sathish, P. Govindaraju, and T. Devaki, "Protective effect of Centella asiatica on antioxidant tissue defense system against adriamycin induced cardiomyopathy in rats," *Life Sci*, vol. 76, no. 5, pp. 585–597, Dec. 2004, doi: 10.1016/j.lfs.2004.09.009.

[127] P. Bunpo *et al.*, "Inhibitory effects of Centella asiatica on azoxymethane-induced aberrant crypt focus formation and carcinogenesis in the intestines of F344 rats," *Food and Chemical Toxicology*, vol. 42, no. 12, pp. 1987–1997, Dec. 2004, doi: 10.1016/j.fct.2004.06.022.

[128] C. Yoosook, N. Bunyapraphatsara, Y. Boonyakiat, and C. Kantasuk, "Anti-herpes simplex virus activities of crude water extracts of Thai Medicinal Plants," *Phytomedicine*, vol. 6, no. 6, pp. 411–419, Jan. 2000, doi: 10.1016/S0944-7113(00)80068-9.

[129] G. K. Jayaprakasha and L. J. M. Rao, "Chemistry, Biogenesis, and Biological Activities of *Cinnamomum zeylanicum*," *Crit Rev Food Sci Nutr*, vol. 51, no. 6, pp. 547–562, Jul. 2011, doi: 10.1080/10408391003699550.

[130] R. Jadeja, M. Thounaojam, Ansarullah, A. V Ramachandran, and R. Devkar, "Phytochemical Constituents and Free Radical Scavenging Activity of Clerodendron glandulosum.Coleb Methanolic Extract," *J Complement Integr Med*, vol. 6, no. 1, Jul. 2009, doi: 10.2202/1553-3840.1226.

[131] R. N. Jadeja *et al.*, "Toxicological evaluation and hepatoprotective potential of Clerodendron glandulosum.Coleb leaf extract," *Hum Exp Toxicol*, vol. 30, no. 1, pp. 63–70, Jan. 2011, doi: 10.1177/0960327110368420.

[132] H. C. Lin, Y.-L. Kuo, W.-J. Lee, H.-Y. Yap, and S.-H. Wang, "Antidermatophytic Activity of Ethanolic Extract from *Croton tiglium*," *Biomed Res Int*, vol. 2016, pp. 1–6, 2016, doi: 10.1155/2016/3237586.

[133] J.-F. Wang, W.-J. He, X.-X. Zhang, B.-Q. Zhao, Y.-H. Liu, and X.-J. Zhou, "Dicarabrol, a new dimeric sesquiterpene from Carpesium abrotanoides L.," *Bioorg Med Chem Lett*, vol. 25, no. 19, pp. 4082–4084, Oct. 2015, doi: 10.1016/j.bmcl.2015.08.034.

[134] S. Remy and M. Litaudon, "Macrocyclic Diterpenoids from Euphorbiaceae as A Source of Potent and Selective Inhibitors of Chikungunya Virus Replication," *Molecules*, vol. 24, no. 12, p. 2336, Jun. 2019, doi: 10.3390/molecules24122336.

[135] Y. Ma *et al.*, "Combination of diethyldithiocarbamate with 12-O-tetradecanoyl phorbol-13-acetate inhibits the growth of human myeloid leukemia HL-60 cells *in vitro* and in xenograft model," *Biosci Biotechnol Biochem*, vol. 84, no. 10, pp. 2069–2076, Oct. 2020, doi: 10.1080/09168451.2020.1789837.

[136] S. Das, P. Kumar, and S. P. Basu, "PHYTOCONSTITUENTS AND THERAPEUTIC POTENTIALS OF DATURA STRAMONIUM LINN," *Journal of Drug Delivery and Therapeutics*, vol. 2, no. 3, May 2012, doi: 10.22270/jddt.v2i3.141.

[137] T. Li, Z. Wei, and H. Kuang, "UPLC-orbitrap-MS-based metabolic profiling of HaCaT cells exposed to withanolides extracted from Datura metel.L: Insights from an untargeted metabolomics," *J Pharm Biomed Anal*, vol. 199, p. 113979, May 2021, doi: 10.1016/j.jpba.2021.113979.

[138] S. Bawazeer and A. Rauf, "In Vitro Antibacterial and Antifungal Potential of Amyrin-Type Triterpenoid Isolated from Datura metel Linnaeus," *Biomed Res Int*, vol. 2021, pp. 1–5, Sep. 2021, doi: 10.1155/2021/1543574.

[139] Z. Qin *et al.*, "Anti-inflammatory active components of the roots of *Datura metel*," *J Asian Nat Prod Res*, vol. 23, no. 4, pp. 392–398, Apr. 2021, doi: 10.1080/10286020.2020.1739660.

[140] "Total withanolides ameliorates imiquimod-induced psoriasis-like skin inflammation".

[141] S. D. Mendelson, "Herbal Treatment of Anxiety: Clinical Studies in Western, Chinese and Ayurvedic Traditions," 2022.

[142] M.-H. Kang, M. S. Lee, M.-K. Choi, K.-S. Min, and T. Shibamoto, "Hypoglycemic Activity of Gymnema sylvestre Extracts on Oxidative Stress and Antioxidant Status in Diabetic Rats," *J Agric Food Chem*, vol. 60, no. 10, pp. 2517–2524, Mar. 2012, doi: 10.1021/jf205086b.

[143] K. S. Jain, M. K. Kathiravan, R. S. Somani, and C. J. Shishoo, "The biology and chemistry of hyperlipidemia," *Bioorg Med Chem*, vol. 15, no. 14, pp. 4674–4699, Jul. 2007, doi: 10.1016/j.bmc.2007.04.031.

[144] Prathyusha M, Rajesh Indala, Anup Jagaralmudi, and Ramesh kumar, "Hepatoprotective Effect of Inularacemosa on Hepatic Ischemia/ reperfusion Induced Injury in Rats," 2013.

[145] B. E. Bastidas Ramírez, N. Navarro Ruíz, J. D. Quezada Arellano, B. Ruíz Madrigal, M. T. Villanueeva Michel, and P. Garzón, "Anticonvulsant effects of Magnolia grandiflora L. in the rat," *J Ethnopharmacol*, vol. 61, no. 2, pp. 143–152, Jun. 1998, doi: 10.1016/S0378-8741(98)00028-2.

[146] K. Watanabe, H. Watanabe, Y. Goto, M. Yamaguchi, N. Yamamoto, and K. Hagino, "Pharmacological Properties of Magnolol and Hōnokiol Extracted from *Magnolia officinalis* : Central Depressant Effects," *Planta Med*, vol. 49, no. 10, pp. 103–108, Oct. 1983, doi: 10.1055/s-2007-969825.

[147] A. M. Clark, A. S. El-Feraly, and W.-S. Li, "Antimicrobial Activity of Phenolic Constituents of Magnolia Grandiflora L.," *J Pharm Sci*, vol. 70, no. 8, pp. 951–952, Aug. 1981, doi: 10.1002/jps.2600700833.

[148] M. W. Feltenstein, W. Schühly, J. E. Warnick, N. H. Fischer, and K. J. Sufka, "Anti-inflammatory and anti-hyperalgesic effects of sesquiterpene lactones from Magnolia and Bear's foot," *Pharmacol Biochem Behav*, vol. 79, no. 2, pp. 299–302, Oct. 2004, doi: 10.1016/j.pbb.2004.08.008.

[149] R. Peñalver, L. Martínez-Zamora, J. M. Lorenzo, G. Ros, and G. Nieto, "Nutritional and Antioxidant Properties of Moringa oleifera Leaves in Functional Foods," *Foods*, vol. 11, no. 8, p. 1107, Apr. 2022, doi: 10.3390/foods11081107.

[150] I. Turel, H. Ozbek, R. Erten, A. Oner, N. Cengiz, and O. Yilmaz, "Hepatoprotective and anti-inflammatory activities of Plantago major L.," *Indian J Pharmacol*, vol. 41, no. 3, p. 120, 2009, doi: 10.4103/0253-7613.55211.

[151] M. Gálvez, "Cytotoxic effect of Plantago spp. on cancer cell lines," *J Ethnopharmacol*, vol. 88, no. 2–3, pp. 125–130, Oct. 2003, doi: 10.1016/S0378-8741(03)00192-2.

[152] M. A. Angarskaya and V. E. Sokolova, “The effect of plantain (Plantago major) on the course of experimental atherosclerosis in rabbits,” *Bull Exp Biol Med*, vol. 53, no. 4, pp. 410–412, Jul. 1963, doi: 10.1007/BF00783859.

[153] R. S. Kumar, T. Sivakumar, R. S. Sundaram, and P. Sivakumar, “Antimicrobial and antioxidant activities of Careya arborea Roxb. stem bark. ,” *Iranian Journal of Pharmacology & Therapeutics*, 2006.

[154] M. Semalty, A. Semalty, A. Badola, G. Joshi, and M. S. M. Rawat, “Semecarpus anacardium Linn.: A review,” *Pharmacogn Rev*, vol. 4, no. 7, p. 88, 2010, doi: 10.4103/0973-7847.65328.

[155] I. Khan *et al.*, “Anti-inflammatory activities of Taxusabietane A isolated from Taxus wallichiana Zucc.,” *Fitoterapia*, vol. 82, no. 7, pp. 1003–1007, Oct. 2011, doi: 10.1016/j.fitote.2011.06.003.

[156] Muhammad Nisar, Inamullah Khan, Shabana Usman Simjee, Anwarul Hasan Gilani, Obaidullah, and Humera Perveen, “Anticonvulsant, analgesic and antipyretic activities of Taxus wallichiana Zucc,” 2008.

[157] H. P. Devkota *et al.*, “Stinging Nettle (Urtica dioica L.): Nutritional Composition, Bioactive Compounds, and Food Functional Properties,” *Molecules*, vol. 27, no. 16, p. 5219, Aug. 2022, doi: 10.3390/molecules27165219.

[158] S. Esposito, A. Bianco, R. Russo, A. Di Maro, C. Isernia, and P. Pedone, “Therapeutic Perspectives of Molecules from Urtica dioica Extracts for Cancer Treatment,” *Molecules*, vol. 24, no. 15, p. 2753, Jul. 2019, doi: 10.3390/molecules24152753.

[159] U K Mazumder, M Gupta, L Manikandan, S Bhattacharya, P K Haldar, and S Roy, “Evaluation of anti-inflammatory activity of Vernonia cinerea Less. extract in rats,” 2003.

[160] E. O. Iwalewa, O. J. Iwalewa, and J. O. Adeboye, “Analgesic, antipyretic, anti-inflammatory effects of methanol, chloroform and ether extracts of Vernonia cinerea less leaf,” *J Ethnopharmacol*, vol. 86, no. 2–3, pp. 229–234, Jun. 2003, doi: 10.1016/S0378-8741(03)00081-3.

[161] Farkaad A Kadir, Normadiah M Kassim, Mahmood A Abdulla, and Wageeh A Yehye, “PASS-predicted Vitex negundo activity: antioxidant and antiproliferative properties on human hepatoma cells-an in vitro study,” 2013.

[162] P. Singh, G. Mishra, S. Srivastava, K. Sangeeta, and R. Khosa, “Phytopharmacological review of Vitex negundo,” 2011.

[163] N. Alam, M. Hossain, M. A. Mottalib, S. A. Sulaiman, S. H. Gan, and M. I. Khalil, “Methanolic extracts of Withania somnifera leaves, fruits and roots possess antioxidant properties and antibacterial activities,” *BMC Complement Altern Med*, vol. 12, Oct. 2012, doi: 10.1186/1472-6882-12-175.

[164] V. S. Sivasankarapillai *et al.*, “Overview of the anticancer activity of withaferin A, an active constituent of the Indian ginseng Withania somnifera,” *Environ Sci Pollut Res Int*, vol. 27, no. 21, pp. 26025–26035, Jul. 2020, doi: 10.1007/S11356-020-09028-0.

[165] Alexey A. Lagunin *et al.*, “CLC-Pred 2.0: A Freely Available Web Application for In Silico Prediction of Human Cell Line Cytotoxicity and Molecular Mechanisms of Action for Druglike Compounds,” 2023.

[166] A. C. Bharti, N. Donato, and B. B. Aggarwal, “Curcumin (diferuloylmethane) inhibits constitutive and IL-6-inducible STAT3 phosphorylation in human multiple myeloma cells,” *J Immunol*, vol. 171, no. 7, pp. 3863–3871, Oct. 2003, doi: 10.4049/JIMMUNOL.171.7.3863.

[167] C. L. Yang *et al.*, “Curcumin blocks small cell lung cancer cells migration, invasion, angiogenesis, cell cycle and neoplasia through Janus kinase-STAT3 signalling pathway,” *PLoS One*, vol. 7, no. 5, May 2012, doi: 10.1371/JOURNAL.PONE.0037960.

[168] H. Y. Kim, E. J. Park, E. Joe, and I. Jou, “Curcumin suppresses Janus kinase-STAT inflammatory signaling through activation of Src homology 2 domain-containing tyrosine

phosphatase 2 in brain microglia," *J Immunol*, vol. 171, no. 11, pp. 6072–6079, Dec. 2003, doi: 10.4049/JIMMUNOL.171.11.6072.

[169] U. P. Singh, O. Prakash, and A. B. Ray, "Antifungal Activity of Withametelin, a Withanolide Isolated from *Datura metel*," *Mycobiology*, vol. 29, no. 2, pp. 96–99, Jun. 2001, doi: 10.1080/12298093.2001.12015768.

[170] P. C. Rao, S. Begum, M. A. F. Jahromi, Z. H. Jahromi, S. Sriram, and M. Sahai, "Cytotoxicity of withasteroids: withametelin induces cell cycle arrest at G2/M phase and mitochondria-mediated apoptosis in non-small cell lung cancer A549 cells," *Tumor Biology*, vol. 37, no. 9, pp. 12579–12587, Sep. 2016, doi: 10.1007/S13277-016-5128-5/METRICS.

[171] A. Khan *et al.*, "Withametelin, a novel phytosterol, alleviates neurological symptoms in EAE mouse model of multiple sclerosis via modulation of Nrf2/HO-1 and TLR4/NF-κB signaling," *Neurochem Int*, vol. 151, p. 105211, Dec. 2021, doi: 10.1016/J.NEUINT.2021.105211.

[172] H. Fatima *et al.*, "Cancer Chemopreventive and Cytotoxic Activities of Isowithametelin from Datura innoxia," *Revista Brasileira de Farmacognosia*, vol. 30, no. 5, pp. 723–728, Oct. 2020, doi: 10.1007/S43450-020-00102-9/METRICS.

[173] D. Han, T. Yu, N. Dong, B. Wang, F. Sun, and D. Jiang, "Napabucasin, a novel STAT3 inhibitor suppresses proliferation, invasion and stemness of glioblastoma cells," *J Exp Clin Cancer Res*, vol. 38, no. 1, Jul. 2019, doi: 10.1186/S13046-019-1289-6.

[174] T. Oashi, A. L. Ringer, E. P. Raman, and A. D. MacKerell, "Automated selection of compounds with physicochemical properties to maximize bioavailability and druglikeness," *J Chem Inf Model*, vol. 51, no. 1, pp. 148–158, Jan. 2011, doi: 10.1021/CI100359A/SUPPL_FILE/CI100359A_SI_001.PDF.

[175] R. Shaltiel-Karyo *et al.*, "A blood-brain barrier (BBB) disrupter is also a potent α-synuclein (α-syn) aggregation inhibitor: a novel dual mechanism of mannitol for the treatment of Parkinson disease (PD)," *J Biol Chem*, vol. 288, no. 24, pp. 17579–17588, Jun. 2013, doi: 10.1074/JBC.M112.434787.

[176] M. Das, C. Mohanty, and S. K. Sahoo, "Ligand-based targeted therapy for cancer tissue," *Expert Opin Drug Deliv*, vol. 6, no. 3, pp. 285–304, Mar. 2009, doi: 10.1517/17425240902780166.

LIST OF PUBLICATIONS AND THEIR PROOFS

Title of Paper - "Navigating Glioblastoma Therapy: A Computational and Drug Repurposing Approach Targeting STAT3"

Author Names- Nancy and Prof. Pravir Kumar

Name of the Conference- 5th International Conference for Emerging Technology (INCET) 2024, Belgaum, India.

Date of Conference- 24th May – 26th May 2024

Indexing- IEEE

Status of Paper- Accepted

Date of Acceptance- 8th March 2024

Date of Camera-Ready Submission and Registration- 28th March 2024 and 15th March 2024

Acceptance Notification - IEEE 5th INCET 2024 External Inbox ×

Microsoft CMT <email@msr-cmt.org>
to me

Dear Nancy Malik

Paper ID / Submission ID : 1001

Title : Navigating Glioblastoma Therapy: A Computational and Drug Repurposing Approach Targeting STAT3

Greeting from 5th INCET 2024.

We are pleased to inform you that your paper has been accepted for the Oral Presentation as a full paper for EMERGING TECHNOLOGIES (INCET), Belagavi, Karnataka, India with following reviewers' comment.

All accepted and presented papers will be submitted to IEEE Xplore for the further publication.

Note:
All of Accepted and Presented Papers of INCET series has been Published by IEEE Xplore and indexed by Scopus
http://www.incet.org/history-incet/

You should finish the registration before deadline, or you will be deemed to withdraw your paper:

Complete the Registration Process (The last date of payment Registration is 15 MARCH 2024)

Payment Links

For Indian Authors: https://rzp.io/l/Afx6l4LaH

Jain College of Engineering
Belagavi, India

IEEE BANGALORE SECTION

5th International Conference of Emerging Technology
(INCET 2024)

24th – 26th May 2024

Certificate

This is to certify that Dr./Prof./Mr./Ms. ***Nancy*** *has presented paper entitled* ***Navigating Glioblastoma Therapy: A Computational and Drug Repurposing Approach Targeting STAT3*** *in 5th International Conference of Emerging Technology (INCET 2024) during 24th to 26th May 2024.*

Dr. Krupa Rasane
Convener - INCET 2024

Dr. J. Shivakumar
General Chair - INCET 2024

Navigating Glioblastoma Therapy: A Computational and Drug Repurposing Approach Targeting STAT3

Nancy
Molecular Neurosciences and Functional Genomics Laboratory, Dept. of Biotechnology
Delhi Technological University
New Delhi – 110042, India
nancy_2k22mscbio32@dtu.ac.in

Prof. Pravir Kumar
Molecular Neurosciences and Functional Genomics Laboratory, Dept. of Biotechnology
Delhi Technological University
New Delhi – 110042, India
pravirkumar@dtu.ac.in

***Abstract*- Glioblastoma, also known as glioblastoma multiforme (GBM) is one of the most formidable and resistant malignancies in humans. Despite the development of multimodality strategies, the success rate remains limited. Consequently, there exists a pressing imperative to discover novel factors that could function as efficacious therapeutic targets for the management of GBM progression. We identified STAT proteins which are signal transducers specifically activated by JAK and Src kinases by phosphorylation. Within the STAT protein family, STAT3 is acknowledged for its oncogenic role and gain-of-function mutations result in its constitutive activation in glioma cells. STAT3 involvements in a wide array of tumorigenesis functions and signaling pathways responsible for malignancies makes STAT3 an attractive target for the treatment of glioblastoma. To find more effective drugs against STAT3 we performed molecular docking using existing FDA-approved drugs and reference drug as control. After docking three potential candidates were identified based on the docking scores. For the validation of these compounds, ADME analysis was performed providing information on about the blood-brain barrier permeability, water solubility, skin permeability, lipophilicity and bioavailability. These findings represent a promising step forward in the pursuit of more efficacious treatments for glioblastoma.**

Keywords— Glioblastoma, STAT, binding energy, blood brain barrier, SwissADME

I. INTRODUCTION

Gliomas are diffusely infiltrative tumors originated from glial cells affecting the surrounding brain tissue. Previously gliomas were classified on the basis of histopathologies into different subtypes and grades for example astrocytoma, oligodendrogliomas or oligoastrocytomas but recently gliomas were classified on the basis of molecular and genetic markers [1]. Gliomas are categorized by the WHO into grade I to IV according to their degree of malignancy, with glioblastoma multiforme (GBM) falling into category IV. GBM is a highly aggressive type of primary astrocytoma occurring frequently. It is a crucial public health issue due to its poor prognosis with survival rate of only 14-15 months after diagnosis [2].

GBM's high permeability, molecular heterogeneity and immune system evasion ability, infiltrative capacity, radio- and chemo-resistance makes it the deadliest of all brain tumors. Despite intense studies and use of multimodality therapies including surgery, radiotherapy and chemotherapy the prognosis and treatment of GBM remain universally poor. The resistance observed in GBM is attributed to specific factors, including blood-brain barrier (BBB), tumor heterogeneity and immunosuppressive tumor microenvironment (TME).

Multiple factors are responsible for tumor development and one of the most important factors is oncogenic signalling pathways which have redundant contributions in the promotion of tumorigenesis and confounding therapeutic strategies. Janus kinase/signal transducers and activators of transcription (JAK/STAT) pathway exhibits activity within critical cellular populations in the microenvironment, including glioma cells, reactive astrocytes, stromal cells and immune cells [3]. This pathway manages the replication, antiapoptotic, angiogenic, and immunosuppressive functions in the tumor microenvironment and progression of tumors. Thus, inhibiting this pathway can be an effective approach to treat GBM [4].

More than 50 cytokines and growth factors are involved in this pathway leading to critical cellular events. It is an evolutionarily conserved pathway consisting of cell surface receptors, JAKs and STATs. The JAK family comprises four members, functioning as non-receptor protein kinases consisting of two domains, a catalytic domain and a kinase-like domain. When the ligand binds to their receptor, JAK tyrosine kinases are activated. Upon activation, JAKs undergo trans-phosphorylation at specific tyrosine residues, creating docking sites that facilitate the recruitment of latent cytoplasmic factors, referred to as STATs.

STATs belongs to the family of transcription factors consisting of seven isoforms. Members of STAT family are composed of 700-950 amino acids with six common domains and the function of each domain is illustrated in Fig. 1 of STAT3 [5].

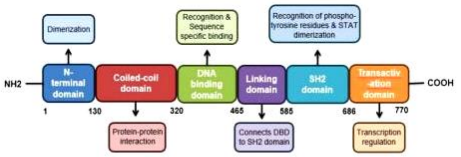


Fig. 1. Linear structure of STAT3 protein containing six domains each serving a specific function.

Upon interaction with cytokines and growth factors with the receptors, these transcription factors are activated by phosphorylation at transactivation domain of key tyrosine residues of various tyrosine kinases such as JAKs and SRC kinases. Subsequently, STAT–STAT dimerization is initiated through reciprocal interaction between phospho-tyrosine (p-

Tyr) and the SH2 domain. This dimerization event subsequently leads to the regulation of gene transcription within the nucleus [6].

A. STAT3

Out of seven members of STAT family, STAT3 mediates various fundamental processes such as immune response, cellular respiration, differentiation, growth, survival, and regeneration. It has been reported that in 50-90% of human cancers, STAT3 exhibits constitutive activation regulated by myriad of signaling pathways implicated in oncogenic processes. These pathways encompassing epidermal growth factor receptor (EGFR), heregulin-2/neuregulin receptor (Her2/Neu), Src tyrosine kinases, c-Met, Abelson leukemia protein (ABL) and others, intricately contribute to constitutive activation of STAT3. Under physiological conditions, STAT3 undergoes intricate regulation by upstream regulatory entities, expressed ubiquitously across diverse cell types. But the disruption of these regulatory molecules can mediate tumorigenesis[7]. The upstream activation signals trigger STAT3 activation through phosphorylation, which mediates the transcription of genes involved in tumor growth, invasion, angiogenesis and immune escape. That phosphorylated STAT3 is aberrantly implicated in the development, metastasis, and creation of tumors, which could have an impact on a patient's clinical prognosis. Lin et al. investigated the expression of phosphorylated STAT3 (p-STAT3) in a cohort of 90 glioblastoma patients, revealing that elevated p-STAT3 expression correlated with reduced progression-free and overall survival years. The subsequent elucidation of five distinct mechanisms, delineated in Fig. 2, identifies STAT3 as a versatile regulator influencing cancer initiation, progression, and metastasis [8].

Fig. 2. An illustrative schematic delineating the intricate association between STAT3 and cancer hallmarks. STAT3 enhances the expression of pivotal tumor hallmarks through five distinct aspects is presented.

Given that p-STAT3 is a molecular hub for multiple glioma signal transduction pathways and is essential to the carcinogenesis and progression of glioblastoma (GBM), it may be a promising target for glioblastoma therapy. Using drug re-purposing approach to find inhibitors of STAT3 can be used in the GBM treatment.

II. METHODOLOGY

A. Protein preparation

The STAT3 with PDB ID of 6TLC was retrieved from Protein Data Bank, a database containing 3D structures of experimentally determined biological macromolecules with a resolution of 2.90 Å in .pdb format by clicking Download Files < PDB Format. Chain A and Chain B along with two MS3-6 were downloaded. (https://www.rcsb.org/structure/6TLC). Through a refined approach using PyMOL, strategic modifications were executed wherein MS3-6 monobodies and Chain A were removed along with non-essential water molecules and the gaps were filled using the sequences available in UniProt (https://www.uniprot.org/uniprotkb/P40763/entry#sequences) for the compatibility with docking software. The prepared protein was saved as a .pdb file following File < Export molecule < Save < Save as type (PDB).

B. Ligand Preparation

For identifying a potential inhibitory drug for STAT3, an FDA-approved drug library containing 3,674 drugs sourced from DrugBank is used for docking purpose. The downloaded ligands were in .sdf format which were opened in Open Babel within Pyrex for conversion from .sdf to .pdbqt format after energy minimization for compatibility with docking software.

C. PyRx

Molecular docking plays a pivotal role in drug discovery by forecasting how potential drug compounds attach to target proteins, aiding in the identification of promising candidates. It streamlines the process of selecting molecules with favorable interactions for subsequent experimental exploration. Tools such as PyRx enhance the efficiency of Docking-Based Virtual Screening across large molecular datasets. It is a virtual screening tool that can be used to screen compound libraries. It makes use of a substantial collection of well-known open-source software. The present study utilizes Open Babel for the importation of ligands and energy minimization and Autodock Vina for the docking process.

D. Protein-Ligand docking

Following preparation, the protein and ligand the next step involves loading of prepared protein into PyRx and then converted into macromolecule. Then the selected ligands were inserted in Open Babel within the software following Minimize Selected < Convert Selected to Autodock ligand (.pdbqt). After preparation both protein and ligands will be visible under the Autodock Tab. Blind docking was performed between the Ligands and Macromolecules chosen using Vina Wizard. The docking site was expanded to its maximum dimensions using the 'maximize' function which can be further adjusted to include the complete protein within the grid box, with final dimensions X = 113.671461449 Å, Y = 72.361182909 Å and Z= 104.551233385 Å. Finally, the binding affinities between the ligand and protein were calculated by using PyRx Vina algorithms. After the docking was completed, the results were saved in .csv and output files at a selected location for 2D interaction analysis for illustration of different types of interactions.

E. ADME analysis

SwissADME is a free online tool that provides the users to access fast and reliable predictive models addressing pharmacokinetics, physicochemical properties, drug-likeness and medicinal chemistry friendliness. This indispensable tool serves as a crucial support system for support drug discovery. Leveraging canonical SMILES for

ligands that met the predefined criteria were extracted from PubChem and employed to scrutinize the various properties of compounds in Swiss ADME.

F. Protein-Ligand interaction visualization

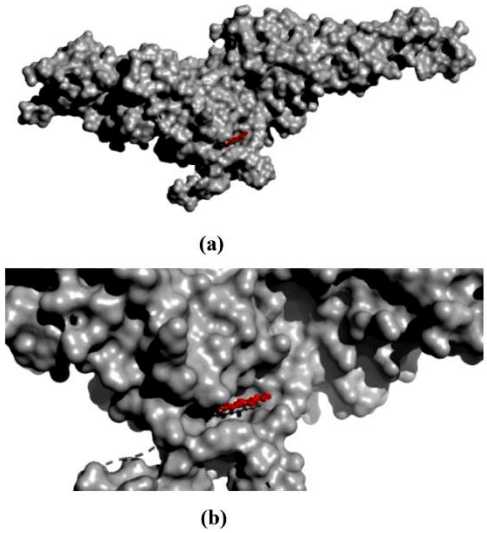
The intricate 2D and 3D interactions between proteins and compounds displaying the highest affinity are visualized with precision using advanced software tools such as Discovery Studio 2021 and PyMOL. These platforms enable comprehensive visualization of the molecular structures and their interactions, allowing for a detailed examination of binding modes, hydrogen bonding, hydrophobic interactions, and other crucial molecular interactions.

III. RESULTS

A. Drug-target and BBB permeability analysis

The FDA-approved drug library was subjected to docking analysis taken from DrugBank. To interpret the potential inhibitor drug napabucasin was taken as a reference drug. Napabucasin interacted with STAT3 with the docking score of -7.5 kcal/mol presented in Fig. 3. The highest binding affinity value of -10 kcal/mol for elbasvir is obtained against the receptor Stat3. Based on the obtained results, we considered drugs with a binding affinity value of -8.5 kcal/mol and higher for BBB permeability analysis using SWISS ADME. BBB permeability analysis revealed that 10 compounds had BBB penetrant activity and were omitted for further analysis. The binding affinity and interactions of these compounds are analyzed using Discovery Studio 2021 indicated in Table I.

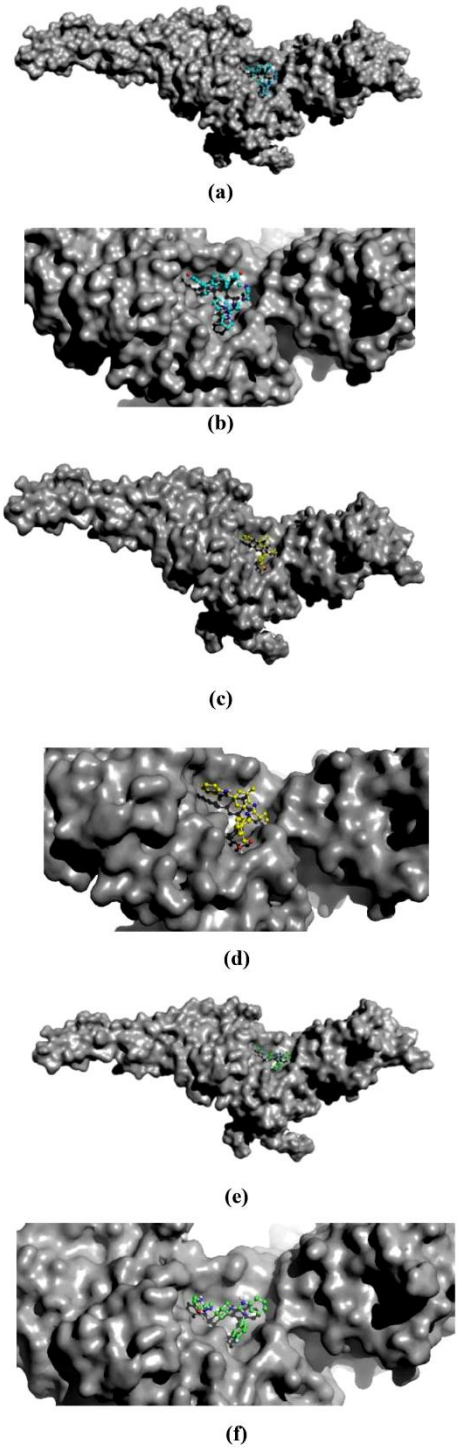
The target-ligand complex's most stable interaction was correlated with the most negative binding energy. Out of selected compounds we observed tirilazad had the most negative energy of -9.5 kcal/mol followed by telmisartan and mizolastine. The 3D interaction of the highest affinity compounds with STAT3 is shown Fig. 4.



(a)

(b)

Fig. 3. (a) 3D structure of complete STAT3 molecule illustrating the specific binding site for napabucasin (b) Enlarged 3D configuration illustrating the binding mode between napabucain and STAT3.



(a)

(b)

(c)

(d)

(e)

(f)

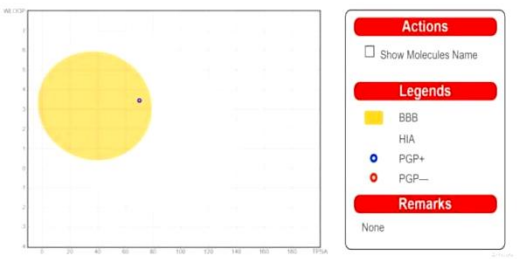
Fig. 4. (a), (c) and (e) 3D configuration of entire STAT3 molecule, elucidating the precise binding position of tirilazad, telmisartan and mizolastine (b), (d) and (f) Enlarged view depicting the molecular interactions and binding mode of tirilazad, telmisartan and mizolastine.

TABLE I. COMPREHENSIVE DOCKING RESULTS OF SELECTED COMPOUNDS IN CONJUNCTION WITH REFERENCE DRUG AND SPECIFYING THE INTERACTING RESIDUES

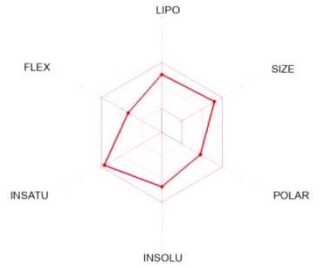
S.No.	Compound	Binding Energy (kcal/mol)	Interacting Residues
1.	Tirilazad	-9.5	Thr346, Gln326, Pro336, Arg325, Cys251, Asp334, Glu324, Cys259, Leu260, Trp243, Asn257, Gln247, Ala250, Pro256, Gly254, Pro333, Pro330, Cys328
2.	Telmisartan	-9.1	Cys328, Thr346, Cys259, Leu260, Glu324, Asn257, Trp243, Gln247, Pro256, Ala250, Cys251, Gly253, Asp334, Ser514, Ile252, Pro333, Met329, Pro330, Arg325, Pro336, Trp474
3.	Mizolastine	-9	Asn257, Pro336, Arg325, Cys328, Pro333, Asp334, Cys251, Ser514, Pro256, Glu324, Leu260, Cys259, Trp243, Gln247, Ala250, Gln326
4.	Apomorphine	-8.9	Asp369, Lys370, Ile368, His437, Leu436, Val490, Arg379, Asn491, Asp371, Leu438, Thr440
5.	Hydroxyestrone diacetate	-8.9	Ser381, Leu430, Asn491, Leu435, Leu436, Pro378, His437, Ser372, Asp369, Val490, Leu438, Thr440, Asp371, Lys370, Glu455, Lys488, His457, Arg379
6.	Bagrosin	-8.8	Ala250, Asp334, Ser514, Pro333, Pro336, Arg325, Gln326, Cys251, Glu324, Gln247, Asn257, Ile252, Gly253, Trp474
7.	Ponatinib	-8.6	Arg308, Ser319, Thr236, Gln232, Lys233, Asp237, Lys244, His457, Thr456, Lys318, Lys488, Asn315, Leu316, Leu312, Glu307, Glu229, Glu311
8.	Mifepristone	-8.6	Gly254, Pro256, Cys328, Pro336, Gln326, Cys251, Gln247, Arg325, Glu324, Cys259, Trp243, Asn257, Ala250, Gly253, Ser514
9.	Elliptinium	-8.6	Asp369, Gly380, Ser381, Leu436, His437, Val490, Lys370, Leu438, Arg379, Asp371
10.	Amitriptylinoxide	-8.5	Ser381, Thr440, Lys370, Leu438, Asp369, Leu436, His437, Glu435, Arg382, Pro378, Val490, Ser372, Arg379, Asn491, Asp371
11.	Napabucasin	-7.5	Ser381, Leu438, Asp371, Thr440, Lys370, Asp369, Arg379, Val490, His437, Leu436

B. ADME analysis

ADME analysis is performed using Swiss ADME to determine the physiochemical and ADME properties of a drug. It provides insight into the physiochemical property values revealing that tirilazad and telmisartan exceeded the threshold while the scores for mizolastine was within the permissible range. Lipinski's rule of five which helps in the prediction of physical and chemical properties of a bioactive molecule to be orally bioavailable is followed by tirilazad and mizolastine with the bioavailability score of 0.55. Significant skin permeability values (Log Kp) and high gastrointestinal (GI) absorption were observed for tirilazad and mizolastine. Lipophilicity is another key factor which is estimated by freely available predictive models in SwissADME. Through this analysis, we identified the characteristics of the top three compounds. Upon analysis, the consensus partition coefficient value (Log Po/w) obtained for tirilazad, telmisartan and mizolastine, were 4.64, 5.98 and 3.28 with poor and moderate solubility. Upon scrutinizing the outcomes across the reported compounds, we observed that mizolastine yielded noteworthy results. The BOILED EGG image of mizolastine is given in Fig. 5.



(a)



(b)

Fig. 5. (a) BIOLED EGG image for mizolastine derived subsequent to ADME analysis (b) Bioavailability radar diagram illustrating a radar plot that distinctly positions mizolastine entirely within the designated pink area.

IV. CONCLUSION

GBM stands as a prevalent primary malignancy characterized by high resistance and recurrence rates, posing challenges to conventional treatment approaches. Resistance mechanisms, including ineffective targeting and the blood-brain barrier, contribute to the difficulty in managing this condition. Recognizing the imperative for novel therapeutic avenues, we identified STAT3 as promising target for therapeutic interventions. This is rooted in its role as a pivotal convergence point for a myriad of oncogenic signaling pathways. In this study we proposed potential inhibitors of STAT3 by performing molecular docking of FDA approved drugs employing napabucasin, a tumor stem cell inhibitor as a reference drug. Using binding energy scores and BBB permeability analysis we found that tirilazad, telmisartan and mizolastine had the most negative ΔG and thus showed the most suitable interaction with the target suggesting it to be an important STAT3 inhibitor.

Based on ADME results, mizolastine exhibits superior pharmacokinetic characteristics compared to two other drug molecules. However, further studies need to be conducted to substantiate the validity of the proposed model.

ACKNOWLEDGMENT

We wish to extend our sincere appreciation to the senior administration of Delhi Technological University (DTU), Department of Biotechnology for their unwavering support.

REFERENCES

[1] F. B. Mesfin, "Gliomas," StatPearls - NCBI Bookshelf, May 20, 2023. https://www.ncbi.nlm.nih.gov/books/NBK441874/

2] D. N. Louis et al., "The 2007 WHO Classification of Tumours of the Central Nervous System," Acta Neuropathol, vol. 114, no. 2, pp. 97–109, Aug. 2007.

[3] A. Ou, M. Ott, D. Fang, and A. Heimberger, "The Role and Therapeutic Targeting of JAK/STAT Signaling in Glioblastoma," Cancers (Basel), vol. 13, no. 3, p. 437, Jan. 2021.

[4] D. Hanahan and R. A. Weinberg, "Hallmarks of Cancer: The Next Generation," Cell, vol. 144, no. 5, pp. 646–674, Mar. 2011.

[5] F. Seif, M. Khoshmirsafa, H. Aazami, M. Mohsenzadegan, G. Sedighi, and M. Bahar, "The role of JAK-STAT signaling pathway and its regulators in the fate of T helper cells," Cell Communication and Signaling, vol. 15, no. 1, p. 23, Dec. 2017.

[6] M. Jain et al., "Role of JAK/STAT in the Neuroinflammation and its Association with Neurological Disorders," Ann Neurosci, vol. 28, no. 3–4, pp. 191–200, Jul. 2021.

[7] J. Kim, M. Patel, J. Ruzevick, C. Jackson, and M. Lim, "STAT3 Activation in Glioblastoma: Biochemical and Therapeutic Implications," Cancers (Basel), vol. 6, no. 1, pp. 376–395, Feb. 2014,.

[8] H. Wang et al., "STAT3 pathway in cancers: Past, present, and future," MedComm (Beijing), vol. 3, no. 2, Jun. 2022.

3rd International Conference

On

"Antimicrobial Resistance, Novel Drug Discovery and Vaccine Development: Challenges and Opportunities"

Organized by

SRM University Delhi - NCR, Sonepat

March 18 – 20, 2024

CERTIFICATE

This is to certify that ..... Nancy ..... has delivered an ~~oral~~ / poster presentation in the 3rd International Conference held on March 18-20, 2024 at India Habitat Centre, New Delhi-110003, India

Prof. (Dr.) V. Samuel Raj
Organizing Secretary

Prof. (Dr.) Paramjit S. Jaswal
Vice-Chancellor

JAMIA HAMDARD
(Deemed to be University)
Accredited in "A+" Grade by NAAC

Society for Neurochemistry, India (SNCI), Delhi Local Chapter

&

Department of Toxicology, School of Chemical and Life Sciences, Jamia Hamdard, New Delhi

Certificate Of Participation

This is to certify that Prof./Dr./Ms./Mr. Nancy of Delhi Technological University has Participate as ***Delegate*** in the Two Days National Symposium on ***"Recent Advances in Neurochemistry and Neurosciences"*** held from 25th April 2024 to 26th April 2024 at Convention Centre, Jamia Hamdard, New Delhi. He/She has also presented in Young Investigator/Poster Session.

Prof. Mohammad Akram
Organizing Secretary

Prof. Suhel Parvez
Organizing Chairperson

Prof. Prakash Babu Phanithi
Secretary General (HQ), SNCI

JAMIA HAMDARD

(Deemed to be University)
Accredited in "A+" Grade by NAAC

One Week STUTI Workshop

Translational Neuroscience: Bridging Gap between Bench to Bedside

Certificate Of Participation

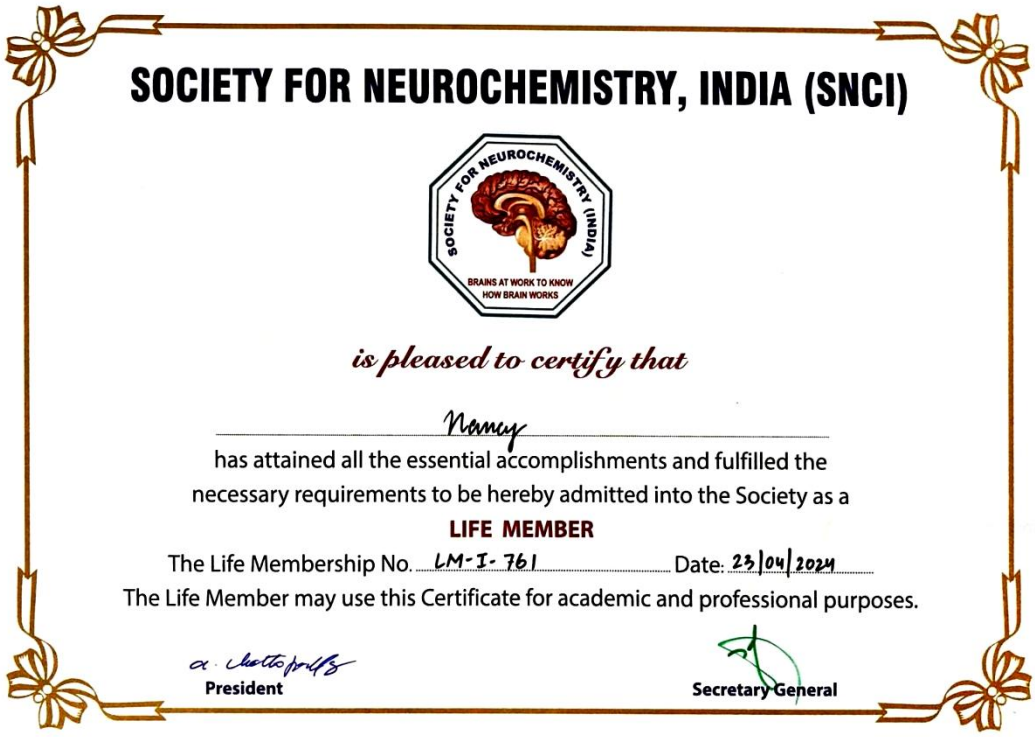
This is to certify that Dr./Ms./Mr. *Ms. Nancy*

of *Delhi Technological University* has contributed as ***Participant*** in the One Week STUTI Workshop on ***"Translational Neuroscience: Bridging Gap between Bench to Bedside"*** held from 18th April 2024 to 24th April 2024 at Jamia Hamdard, New Delhi

Prof. Mohammad Akram
Organizing Secretary

Prof. Suhel Parvez
Organizing Chairperson

Prof. Prakash Babu Phanithi
Secretary General (HQ), SNCI



SOCIETY FOR NEUROCHEMISTRY, INDIA (SNCI)

is pleased to certify that

Nancy

has attained all the essential accomplishments and fulfilled the necessary requirements to be hereby admitted into the Society as a

LIFE MEMBER

The Life Membership No. LM-I-761 Date: 23/04/2024

The Life Member may use this Certificate for academic and professional purposes.

President

Secretary General

DELHI TECHNOLOGICAL UNIVERSITY
(Formerly Delhi College of Engineering)
Shahbad Daulatpur, Main Bawana Road, Delhi-110042

PLAGIARISM VERIFICATION

Title of Thesis Synergizing In-Silico Approaches of Natural Product-based Drug Discovery and Drug Repurposing to target STATs in Glioblastoma Therapeutics

Total Pages 50 Name of the Student Manu

Supervisor Prof. Pravir Kumar

Department Biotechnology

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

Software used: Turnitin Similarity Index: 5% Total Word Count: 14403

Date: 4 June 2024

Candidate's Signature

05/06/2024

Signature of Supervisor

Plagiarism Report

Similarity Report

PAPER NAME **Nancy thesis docx.pdf**	AUTHOR **N**
WORD COUNT **14403 Words**	CHARACTER COUNT **83440 Characters**
PAGE COUNT **50 Pages**	FILE SIZE **1.6MB**
SUBMISSION DATE **Jun 3, 2024 2:09 PM GMT+5:30**	REPORT DATE **Jun 3, 2024 2:11 PM GMT+5:30**

5% Overall Similarity

The combined total of all matches, including overlapping sources, for each database.

- 4% Internet database
- 2% Publications database
- Crossref database
- Crossref Posted Content database
- 3% Submitted Works database

Excluded from Similarity Report

- Bibliographic material
- Quoted material
- Cited material
- Small Matches (Less then 10 words)

Malik 05/06/24

05/06/24

Summary

CURRICULUM VITAE

NANCY

+91 7053480126 nancymalik918@gmail.com

2K22/MSCBIO/32

EDUCATION

Name of Course	Year	School/College	Grade
M.Sc. (Biotechnology)	2022-2024	Delhi Technological University	
B.Sc. (Microbiology)	2019-2022	University of Delhi, New Delhi	86.013%
CBSE (Class XII)	2019	Rao Mohar Singh Public School	92.2%
CBSE (Class X)	2017	The Dev Public School	95%

WORK EXPERIENCE

- Participated in one week STUTI Workshop on "Translational Neuroscienece: Bridging Gap between Bench to Bedside" from April 18 - April 24, 2024 organised by Department of Toxicology, School of Chemical and Life Sciences, Jamia Hamdard, New Delhi and Society for Neurochemistry, India (SNCI), Delhi Local Chapter.
- Training on "Basic techniques in Molecular biology and Biophysics" at Jamia Millia Islamia from June 8 to August 9, 2023.
- Participated in the "National Workshop on Immuno-Biology Techniques and Their Applications" held from February 13 - February 25, 2023 organised by Centre for Innovation in Infectious Disease Research, Education and Training (CIIDRET) University of Delhi South Campus and Delhi School of Skill Enhancement and Entrepreneurship Development (DSSEED-IoE), University of Delhi.
- Two months laboratory training at Divya Parshtha Hospital from July 1- August 30, 2022.
- Attended "National Workshop on Techniques for Manipulation of Nucleic Acids for Application in Genomics" organised by DBT-Supported Genomics Facility, Centre for Innovation in Infectious Disease Research, Education and Training

(CIIDRET) University of Delhi South Campus and Delhi School of Skill Enhancement and Entrepreneurship Development (DSSEED-IoE), University of Delhi from March 11 - March 21, 2022.

- A four-week online summer training on "Research Methodology" organized by the Department of Biochemistry, Shivaji College, University of Delhi, under the aegis of DBT sponsored Star College Scheme from June 21- July 20, 2021.

ACADEMIC ACHIEVEMENTS AND AWARDS

- Secured 2nd position in Annual Examination held in 2019-20

POSITIONS OF RESPONSIBILITY

- Member of editorial board in Sukshmjeev Society, Department of Microbiology

CONFRENCE

- Attended and presented poster in Two Days National Symposium on "Recent Advances in Neurochemistry and Neurosciences" held from April 25 - April 26, organised by Department of Toxicology, School of Chemical and Life Sciences, Jamia Hamdard, New Delhi and Society for Neurochemistry, India (SNCI), Delhi Local Chapter.
- Attended and presented poster in "3rd International Conference on Antimicrobial Resistance, Novel Drug Discovery and Vaccine Development: Challenges and Opportunities" held on March 18 - March 20, 2024.
- Participated and presented paper titled "Navigating Glioblastoma Therapy: A Computational and Drug Repurposing Approach Targeting STAT3" at 5th International Conference for Emerging Technology (INCET) 2024 technically co-sponsored by IEEE Bangalore Section, Belgaum, India organised at Jain College of Engineering.

OTHER INFORMATION

https://drive.google.com/drive/folders/1GfDeOYFdUg6EQUGMszli03L2BA35AqgW?usp=share_link