Structure-based *In-silico* Identification of Plant-derived Inhibitors Targeting *Prp8* Intein Splicing in *Cryptococcus neoformans*: A Phytochemical Derived Remedy for Antimycotic Drug Resistance

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ANJALI TIWARI
2K23/MSCBIO/08

Under the Supervision of
Dr. NAVNEETA BHARADVAJA

Department of Biotechnology

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

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ANJALI TIWARI

2K23/MSCBIO/08

DELHI TECHNOLOGICAL UNIVERSITY

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

DECLARATION BY THE CANDIDATE

I, **ANJALI TIWARI** (Roll Number: **2K23/MSCBIO/08**), hereby certify that the dissertation entitled **"Structure-based In-silico Identification of Plant-derived Inhibitors Targeting Prp8 Intein Splicing in *Cryptococcus neoformans*: A Phytochemical Derived Remedy for Antimycotic Drug Resistance"**, is the outcome of my own original research. It is submitted in partial fulfilment of the requirements for the Master of Science in the Department of Biotechnology at Delhi Technological University. This work was carried out independently under the guidance of **Dr. Navneeta Bharadvaja** between May 2024 and May 2025.

To the best of my knowledge, the content incorporated in this thesis has not been submitted, in part or in full, for the award of any other degree or diploma at this or any other institution or university.

I take full responsibility for the authenticity and integrity of the work presented herein.

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DELHI TECHNOLOGICAL UNIVERSITY

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

CERTIFICATE

This is to certify that **Ms. ANJALI TIWARI (Roll No. 2K23/MSCBIO/08)** has undertaken and successfully completed the research work embodied in the present dissertation entitled **"Structure-based In-silico Identification of Plant-derived Inhibitors Targeting Prp8 Intein Splicing in *Cryptococcus neoformans*: A Phytochemical Strategy to Overcome Antifungal Drug Resistance,"** submitted in partial fulfilment of the requirements for the award of the degree of **Master of Science** in the Department of Biotechnology, Delhi Technological University, Delhi.

The work presented in this thesis is the outcome of original and independent research conducted by the candidate under my supervision. To the best of my knowledge, the content of this dissertation has not been submitted, either in part or in full, for the award of any other degree or diploma at this or any other University/Institution.

Place: Delhi
Date: 05.06.2025

Dr. Navneeta Bharadvaja
Associate Professor
Supervisor
Dept. of Biotechnology
Delhi Technological University

Prof. Yasha Hasija

H.O.D
Dept. of Biotechnology
Delhi Technological University

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ANJALI TIWARI

tanjali243@gmail.com

ABSTRACT

Cryptococcus neoformans, an encapsulated basidiomycetous yeast, is a clinically significant opportunistic fungal pathogen responsible for cryptococcosis—a disseminated, often fatal systemic mycosis primarily affecting individuals with weaker immune defences, such as people with HIV/AIDS infections or recipients of immunosuppressive regimens. Despite the readily available antifungal agents, the current remedial arsenal is increasingly compromised by escalating resistance, limited target specificity, adverse host toxicity, and poor pharmacokinetic profiles. These limitations underscore an urgent demand for novel antifungal strategies that are both selective and mechanistically innovative.

In this context, the intein embedded within the highly conserved Prp8 protein of the spliceosome represents a compelling molecular target. The Prp8 intein is a self-catalysing protein segment that undergoes autocatalytic protein splicing, a process crucial for the functional maturation of Prp8. Notably, inteins are evolutionarily restricted to unicellular microorganisms and are entirely absent in metazoan proteomes, making them ideal candidates for selective antifungal intervention with minimal risk of off-target effects in human hosts.

This study undertakes a structure-based virtual screening approach targeting the *C. neoformans* Prp8 intein, utilizing its crystallographically resolved structure (PDB ID: 6MX6). A panel of ten phytochemicals, selected based on their redox activity, electrophilic functional groups, and established pharmacological safety, were computationally screened for their potential to inhibit intein splicing. The synthetic intein inhibitor 6G-319S was employed as a reference control to benchmark binding performance.

Among the screened natural compounds, **curcumin** exhibited the highest binding affinity value (−8.7 kcal/mol), outperforming the chosen reference inhibitor (−7.9 kcal/mol), and formed stable interactions with catalytically critical residues such as Cys1 and Asn198, essential for the splicing mechanism. Other top-performing phytochemicals, including **Withaferin A**, **Epigallocatechin gallate (EGCG)**, and **Tinosporaside**, demonstrated substantial binding affinities and engaged in strategic interactions with residues constituting the intein's catalytic core and conserved β-strands, suggesting their potential as allosteric or competitive inhibitors of intein activity.

The present findings substantiate the therapeutic promise of intein-targeted antifungal strategies and demonstrate the efficacy of phytocompound scaffolds as viable chemical entities for modulating microbial-specific post-translational processes. By harnessing the evolutionary

exclusivity of inteins and the structural diversity inherent to plant-derived compounds, this approach introduces a novel, host-sparing, resistance-evading therapeutic paradigm for combating invasive fungal diseases. These insights may lead to the rational advancements in next-generation antifungal agents targeting cryptic molecular mechanisms unique to pathogenic fungi.

Keywords: *Cryptococcus neoformans*, Prp8 intein, intein splicing, antifungal resistance, virtual screening, phytochemicals, post-translational modification, molecular docking, structure-based drug design, curcumin, spliceosome, fungal pathogenesis.

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LIST OF ABBREVIATIONS

PDB	Protein Data Bank
PyRx	Python Prescription
ADME	Absorption, Distribution, Metabolism, and Excretion
BBB	Blood-Brain Barrier
P-gp	P-glycoprotein
SAR	Structure–Activity Relationship
EGCG	Epigallocatechin Gallate
GI	Gastrointestinal
RMSD	Root Mean Square Deviation
3D	Three-Dimensional
2D	Two-Dimensional
kcal/mol	Kilocalories per mole
BOILED Egg	Brain Or IntestinaL EstimateD permeation model
PK/PD	Pharmacokinetics / Pharmacodynamics
Hetatoms	Heteroatoms
BIOVIA DS	BIOVIA Discovery Studio
PDB ID	Protein Data Bank Identification Code
Log P	Logarithm of the Partition Coefficient (octanol/water)
TPSA	Topological Polar Surface Area
MW	Molecular Weight
SAS	Synthetic Accessibility Score
PSA	Polar Surface Area
RTB	Rotatable Bonds
LE	Ligand Efficiency
VDW	Van der Waals

CHAPTER 1

INTRODUCTION

1.1 BACKGROUND

Cryptococcosis remains a formidable global health challenge, particularly among immunocompromised individuals such as those with HIV/AIDS, organ transplant recipients, and patients undergoing chemotherapy or long-term immunosuppressive treatment [1]. The causative agent, *Cryptococcus neoformans*, is a neurotropic, encapsulated fungal pathogen responsible for life-threatening meningoencephalitis and disseminated systemic infections [2]. Despite the longstanding clinical use of antifungals like amphotericin B, flucytosine, and fluconazole, the therapeutic landscape is constrained by increasing antifungal resistance, high host toxicity, poor pharmacokinetics, and limited global accessibility to critical medications [3].

These limitations underscore an urgent need to develop novel antifungal strategies that are both selective and less toxic. A promising novel approach in antimycotic drug discovery targets molecular components that are exclusive to fungal pathogens and not found in human cells. One such target is the Prp8 intein- a self-excising protein segment located within the conserved core of the Prp8 protein in the spliceosome of *Cryptococcus neoformans* [4]. This intein is essential for the correct processing of Prp8, a key player in RNA splicing and gene expression regulation. Its excision, through a conserved series of chemical reactions beginning with a nucleophilic attack and ending with the ligation of the flanking protein segments (exteins), is required to restore proper spliceosome activity [5].

Prp8 intein is an especially attractive target because of its evolutionary uniqueness. Inteins occur widely in microbial organisms but are absent in complex eukaryotes such as humans [6]. The analogous PRPF8 protein in humans does not contain inteins and does not undergo post-translational splicing, presenting an opportunity to selectively disrupt fungal functions without impacting the host. Blocking intein splicing results in impaired Prp8 function, spliceosome failure, deregulated gene expression, and eventually fungal cell death or reduced pathogenicity [7].

In this setting, phytochemicals which are naturally derived bioactive compounds from medicinal plants have garnered interest because of their structural versatility and diversity, low toxicity, and ability to act on multiple targets. Some phytochemicals inhibit intein activity by targeting nucleophilic amino acids, binding essential metal ions, or altering redox balance. Phytocompounds such as Curcumin, EGCG, Nimbin have shown inhibitor effects similar to the synthetic intein inhibitors such as 6G-318S and Cisplatin [8][9], often working as reversible modifiers of cysteine residues or zinc chelators that disrupt intein catalytic function.

The use of computational techniques such as molecular docking followed by molecular dynamics simulations has significantly accelerated the search for effective inhibitors [10].

Structure-based virtual screening provides a rapid and cost-efficient way to identify phytochemicals with strong binding and favourable interactions with fungal proteins [11][12].

This dissertation focuses on the in-silico structure-based identification of plant-derived inhibitors targeting the Prp8 intein splicing in *Cryptococcus neoformans*. By combining fungal-specific molecular targets, phytochemical bioactivities, and computational screening, this research aims to contribute to the development of selective, host-friendly antifungal drugs to address emerging resistance issues.

1.2 SIGNIFICANCE OF THE STUDY

Cryptococcosis remains a formidable opportunistic fungal infection, particularly afflicting individuals with compromised immune systems, such as those with HIV/AIDS, those who have undergone organ transplants, and patients receiving immunosuppressive therapies. The causative fungi, *Cryptococcus neoformans* and *Cryptococcus gattii*, exhibit strong neurotropism, often leading to severe central nervous system involvement, most notably cryptococcal meningoencephalitis—the most critical and potentially fatal manifestation [13]. Although current antifungal agents like amphotericin B, fluconazole, and flucytosine form the cornerstone of treatment, their clinical utility is increasingly constrained by issues such as drug resistance, adverse toxicity profiles, limited spectrum of activity, and unequal global access [14]. These persistent challenges necessitate the exploration and development of novel antimycotic interventions that offer enhanced specificity, reduced toxicity, and broader applicability to improve patient outcomes in the management of cryptococcal disease.

An innovative antimycotic intervention involves uniquely targeting the intein domain embedded within the Prp8 protein of *Cryptococcus neoformans*, an essential component of its spliceosomal machinery [15]. Inteins are self-excising protein elements that undergo precise post-translational excision, seamlessly joining the surrounding exteins to restore full protein functionality [5]. This splicing event is crucial for the structural and functional integrity of the fungal spliceosome, ensuring accurate RNA maturation [5]. Significantly, inteins are absent in the human counterpart of Prp8, offering a highly selective therapeutic window. Pharmacological inhibition of this intein disrupts the maturation of Prp8, thereby destabilizing spliceosome assembly, impairing RNA splicing, and curbing fungal proliferation—all while leaving the host's cellular machinery untouched [16].

Within this therapeutic framework, phytochemicals—bioactive secondary metabolites derived from medicinal plants—emerge as highly promising candidates for intein inhibition. These natural compounds exhibit extensive chemical diversity and bioactivity, including redox modulation, electrophilic interactions, and metal ion chelation, all of which are mechanistically compatible with intein inhibition. Several phytochemicals have been shown to emulate the behaviour of synthetic intein inhibitors. For instance, curcumin and epigallocatechin gallate (EGCG) exhibit strong Zn^{2+}-chelating properties, while allicin, sulforaphane, and withaferin A can covalently modify nucleophilic cysteine residues within catalytic cores. Other compounds like berberine, thymoquinone, and ginkgolides exert oxidative stress or disrupt cysteine redox homeostasis, mimicking the biochemical perturbations induced by compounds such as 6G-318S and cisplatin.

This convergence of pharmacophoric functionality between plant-derived and synthetic inhibitors highlights the underexplored yet potent capacity of phytocompounds to function as selective modulators of intein activity. Given their favourable pharmacokinetics, minimal toxicity, and evolutionary refinement, phytochemicals represent an untapped reservoir of antifungal agents tailored for specificity against fungal-specific molecular pathways.

The integration of in-silico methodologies, such as molecular docking followed by virtual screening and visualisations, enables a rational, structure-based interrogation of the binding potential of phytochemicals to intein catalytic domains [17]. This computational paradigm not only accelerates the early-phase discovery pipeline but also facilitates precision targeting by modelling molecular interactions at atomic resolution.

Hence, this study aims to harness the dual advantage of evolutionary molecular divergence and plant-derived chemical intelligence to identify novel, selective, and biocompatible inhibitors of Prp8 intein splicing in *Cryptococcus neoformans* [18]. The findings hold promise for redefining antifungal therapy through a mechanistically distinct, phytochemical-based modality that transcends the limitations of current antifungal pharmacotherapy.

1.3 STUDY OBJECTIVES

The overarching goal of this analysis is to identify and then characterize phytochemical-based inhibitors that selectively target the intein domain of the Prp8 protein in *Cryptococcus neoformans* through computational structure-based approaches. This study is designed to bridge the disciplines of fungal molecular biology, natural product chemistry, and in silico drug discovery to facilitate the development of evolutionarily selective antifungal therapeutics. The fact-finding specific objectives are as follows:

- To characterize the structural and catalytic landscape of the Prp8 intein domain in *Cryptococcus neoformans*, focusing on the mechanistic relevance of its conserved nucleophilic residues (Cys, Ser, Thr, Asn) and its role in post-translational protein maturation essential for spliceosomal function.
- To curate a rationally selected library of phytocompounds with documented chelating, redox-modulatory, or electrophilic activity—attributes mechanistically aligned with intein splicing inhibition—and to assess their drug-likeness and pharmacological profiles.
- To perform high-precision molecular docking simulations using the crystallographic structure of the *C. neoformans* Prp8 intein (PDB ID: 6MX6) to evaluate phytochemical binding affinities, spatial complementarity, and interaction dynamics with the intein active site, particularly the conserved catalytic core.
- To benchmark the top-ranking phytocompounds against a known intein inhibitor (6G-318S), analysing key parameters such as binding energy scores, hydrogen bonding, hydrophobic contacts, and coordination with metal ions or catalytic residues, in order to identify structurally and energetically favourable leads.
- To propose structurally validated phytochemical inhibitors as potential therapeutic candidates for further experimental validation, supported by molecular interaction maps and comparative docking profiles that underscore their splicing-disruptive potential.
- To contextualize the clinical and therapeutic implications of intein-targeting phytochemicals, highlighting their prospective role in overcoming antifungal resistance, minimizing host toxicity, and enabling broad-spectrum application through combinatorial regimens or formulation into existing treatment protocols.

- To establish an evolution-informed, host-sparing antifungal drug development framework, leveraging the absence of inteins in human proteomes and the ecological intelligence of plant secondary metabolites to guide the next generation of antifungal discovery.

CHAPTER 2

LITERATURE REVIEW

2.1 OVERVIEW OF *C. neoformans* AND CRYPTOCOCCOSIS

Cryptococcus neoformans is a highly adapted, encapsulated basidiomycetous yeast of profound clinical relevance, recognized as the principal etiological agent of cryptococcosis—a severe and often life-threatening systemic mycosis [19]. Phylogenetically assigned to the phylum *Basidiomycota*, class *Tremellomycetes*, and order *Tremellales*, this opportunistic fungal pathogen has garnered global prominence for its neurotropic predilection, particularly in immunocompromised beings such as people carrying HIV/AIDS infections, having received organ transplant, and patients which are undergoing sustained immunosuppressive or cytotoxic treatment regimens.

TAXONOMIC RANK	CLASSIFICATION
Domain	Eukaryota
Kingdom	Fungi
Phylum	Basidiomycota
Subphylum	Agaricomycotina
Class	Tremellomycetes
Order	Tremellales
Family	Tremellaceae
Genus	*Cryptococcus*
Species	***Cryptococcus neoformans***

Table 1: Taxonomic Classification of ***Cryptococcus neoformans***

2.1.1 Ecological Distribution and Mode of Transmission

Cryptococcus neoformans is a globally disseminated, saprophytic basidiomycetous yeast that demonstrates a pronounced ecological predilection for nitrogen-rich substrates, particularly avian excreta (notably pigeon droppings), decomposing lignocellulosic material, and organically enriched soil matrices [20]. It predominantly exists in a unicellular haploid state under ambient environmental conditions but exhibits remarkable reproductive plasticity, undergoing both heterothallic and homothallic (unisexual) mating pathways that yield desiccation-resistant, infectious basidiospores, particularly under nutrient-depleted or environmentally stressed conditions. Human acquisition of infection typically occurs via the inhalation of airborne propagules, which upon deposition in the pulmonary alveoli, may establish an initial infection that remains clinically silent or quiescent in immunocompetent individuals. However, in the context of malfunctioned cell-mediated immunity—such as those with AIDS, recipients of organ transplants, or those undergoing immunosuppressive chemotherapy—the pathogen exhibits the capacity for immune evasion, hematogenous or bloodstream mediated dissemination, and translocation across the BBB (blood–brain barrier),

ultimately precipitating cryptococcal mediated inflammation of the brain, a life-threatening manifestation characterized by profound neuroinflammation and high mortality.

2.1.2 Cryptococcosis: Clinical Spectrum and Pathogenesis

Cryptococcosis is typified by a biphasic trajectory of infection, commencing as a primary pulmonary insult and, in predisposed individuals, culminating in central nervous system (CNS) dissemination [21]. The clinical manifestations are heterogenous, spanning from asymptomatic pulmonary colonization and subacute pneumonitis to life-threatening meningoencephalitis [55][57]. Neurological involvement is frequently marked by cephalalgia, pyrexia, photophobia, nuchal rigidity, and cognitive disturbances, which may escalate to elevated intracranial pressure, neurological deficits, and coma in severe presentations. Moreover, extrapulmonary dissemination through hematogenous routes can extend to dermal tissues, skeletal structures, the prostate, and adrenal glands, underscoring the pathogen's potential for systemic invasiveness and clinical complexity [21].

The pathogenicity of *Cryptococcus neoformans* is attributable to several evolutionarily conserved, multifaceted pathogenic determinants:

- **Polysaccharide Capsule**: *Cryptococcus neoformans* is enveloped by a voluminous, morphologically adaptable polysaccharide containing capsule, mainly constituted of GXM i.e. glucuronoxylomannan and GalXM i.e. galactoxylomannan. This capsule serves as a principal virulence attribute by impairing phagocytic recognition and uptake, and by modulating host immune dynamics through the attenuation of cytokine signaling cascades and disruption of antigen presentation [22]. In addition, capsular polysaccharides are actively shed into host tissues, further enhancing immune subversion and facilitating persistent colonization and dissemination, thereby significantly amplifying the organism's pathogenic potential.

- **Melanin Production**: In *Cryptococcus neoformans*, melanin is synthesized via a laccase-dependent oxidative pathway, wherein phenolic substrates undergo enzymatic polymerization and are integrated into the cell wall matrix [23]. This pigment serves as a critical virulence determinant, affording robust protection against reactive oxygen species (ROS), host-derived immune pressures, and the cytotoxic effects of antifungal agents. Furthermore, melanin pigment also promotes iron acquisition by binding and storing this vital micronutrient under scarce conditions, and imparts heat resistance, thereby improving the pathogen's ability to endure and thrive well within the challenging and unfavorable host ambience [24].

- **Morphogenesis of Titan Cells:** *Cryptococcus neoformans* possesses a unique capability to transform morphologically within the host, generating polyploid titan cells which are substantially enlarged and highly resilient variants that evade phagocytic clearance. These morphotypes function as an immune evasion strategy, providing protective advantages to their descendants and thereby supporting prolonged fungal survival and persistence in the host environment [25].
- **Enzymatic Virulence Weaponry:** The virulence of C. neoformans is further enhanced by various secreted enzymes such as urease, phospholipase B1, and multiple proteases. These enzymes promote invasion of host tissues, compromise cellular integrity, and

enable the pathogen to cross vital anatomical barriers, particularly the BBB or the blood-brain barrier, thereby facilitating the onset of meningitis and encephalitis [21].

- **Thermotolerance and Environmental Stress Adaptation:** Adaptation to mammalian physiological temperatures—particularly the ability to proliferate at 37°C and survive febrile episodes—is integral to fungal virulence. This thermotolerance is underpinned by a highly evolved stress response network, comprising heat shock proteins such as Hsp90, and membrane remodelling mechanisms that safeguard cellular integrity against thermal, oxidative, and osmotic stressors encountered during host infection [26].

2.1.3 Immune Evasion and Intracellular Persistence

Cryptococcus neoformans exhibits a highly evolved repertoire of immune-evasive strategies, enabling it to persist within host cells and disseminate systemically [27]. Upon inhalation and subsequent recognition by alveolar macrophages, the pathogen is internalized via phagocytosis. Rather than being neutralized, *C. neoformans* subverts canonical phagolysosomal maturation processes, allowing it to survive and proliferate within a hostile intracellular milieu characterized by reactive oxygen and nitrogen species. This facultative intracellular lifestyle not only shields the fungus from extracellular immune defences but also facilitates extrapulmonary migration, notably via the "Trojan horse" mechanism—where infected macrophages serve as mobile reservoirs that ferry the pathogen across endothelial barriers, including the blood–brain barrier [28] [29].

The host mounts a predominantly T-helper 1 (Th1)-polarized immune response, orchestrated by cytokines such as IFN-γ or interferon-gamma, TNF-α or the tumour necrosis factor-alpha, and IL-12 or interleukin-12, which are critical for macrophage mobilisation and fungal containment [63]. However, *C. neoformans* employs sophisticated immunomodulatory mechanisms to subdue these responses. The shedding of its polysaccharide capsule interferes with antigen recognition and promotes immune tolerance; it can induce apoptosis of T lymphocytes, thereby depleting cellular effectors; and it impairs dendritic cell maturation and antigen presentation, disrupting the initiation of adaptive immunity.

Collectively, these strategies enable *C. neoformans* to establish chronic infections, evade immunological clearance, and persist in latent reservoirs. In immunologically retarded hosts, particularly hosts with defective cell-mediated immunological response, such adaptations contribute to the high relapse rates and difficulty in achieving sterilizing immunity, underscoring the need for innovative therapeutic strategies targeting fungal-specific pathways [63].

2.2 OVERVIEW OF INTEIN SPLICING

Inteins, also known as intervening protein domains, are unique self-splicing polypeptide sequences that facilitate a specialized form of modifications done post-translationally which are termed as protein splicing [30]. This mechanism, distinct from proteolytic cleavage or RNA splicing, involves the intein excising itself precisely from the host polypeptide and concurrently joining the adjacent flanking extein segments. The result is the generation of a nature, biologically active protein, achieved through a finely regulated, enzyme-independent process [30]. Notably, intein splicing occurs independently of auxiliary enzymes, proceeding via a highly conserved intramolecular mechanism. The evolutionary conservation, biochemical precision, and structural autonomy of this process underscore its significance in

protein evolution and highlight its possibility as novel targeted therapeutics in pathogenic systems that uniquely harbor inteins [34].

2.2.1 Molecular Functioning of Protein Splicing

Protein splicing is an intrinsically orchestrated, auto or self-catalytic post-translational mechanism governed by a precisely choreographed series of nucleophilic substitution reactions [30]. This mechanism is executed by conserved catalytic residues encoded within the intein domain and proceeds through four discrete biochemical transitions that conclude in the deletion of the intein and joining of the surrounding flanking extein sequences to yield a mature, functionally active polypeptide [36][58].

The process is initiated by the nucleophilic attack of a side chain—typically a cysteine, serine, or threonine—located at the intein's N-terminus. This residue catalyses the rearrangement of the peptide bond linking the upstream extein to the intein, forming a reactive (thio)ester intermediate at the junction [31]. Subsequently, a second nucleophile residing at the first residue of the downstream extein engages in a transesterification reaction, targeting the acyl intermediate. This results in the formation of a branched intermediate that transiently joins the two exteins while still tethered to the intein. A highly conserved asparagine at the intein's C-terminal end then undergoes spontaneous cyclization. This step cleaves the peptide bond connecting the intein to the C-terminal extein and facilitates intein excision, leaving the joined exteins in a non-canonical linkage [16] [31]. Finally, the transient (thio)ester bond connecting the two exteins undergo an S-to-N acyl shift, regenerating a conventional amide (peptide) bond and restoring backbone integrity. The product is a ligated extein polypeptide indistinguishable from a ribosomally synthesized continuous protein [31].

This elegant splicing cascade is inherently self-sufficient, energetically neutral, and proceeds under physiological conditions without the assistance of external enzymes, cofactors, or ATP. The kinetics and fidelity of the reaction are context-dependent, being modulated by the identity of flanking extein residues, tertiary protein structure, redox potential, pH, and the availability of metal ions. Such mechanistic precision underscores the intein's utility as both a molecular evolutionary relic and a promising therapeutic target for selective inhibition in pathogenic organisms [36].

2.3 CURRENT TREATMENT OPTIONS FOR CRYPTOCOCCOSIS

Cryptococcosis, primarily instigated by *Cryptococcus neoformans*, continues to represent a formidable opportunistic fungal infection, especially within immunologically vulnerable populations such as people with AIDS, organ transplant recipients, and patients under prolonged immunosuppressive regimens. The pathogen's predilection for the CNS i.e. central nervous system frequently manifests as cryptococcal meningoencephalitis—an often-fulminant clinical syndrome marked by considerable neurological morbidity and a persistently high mortality burden despite antifungal intervention [12][13]. Existing pharmacotherapeutic regimens, notably those comprising amphotericin B formulations, flucytosine, and fluconazole, form the cornerstone of standard treatment [50]. However, these agents are encumbered by substantial clinical limitations, including dose-limiting toxicities (e.g., nephrotoxicity, bone marrow suppression), variable fungicidal efficacy in the CNS milieu, and the growing threat of antifungal resistance. Furthermore, logistical barriers to access in low-

resource settings exacerbate global treatment inequities [50]. Collectively, these challenges highlight an urgent imperative to explore innovative antifungal strategies with improved selectivity, tolerability, and pathogen specificity. In this context, the exploitation of intein-mediated protein splicing—an essential process absent in humans—offers a highly selective molecular target. The advancement of such intein-targeting approaches, exemplified by investigational agents such as the compound 6G-318S, heralds a novel therapeutic frontier in the treatment of cryptococcosis.

2.4 INVESTIGATIONAL THERAPIES: SIGNIFICANCE OF INTEIN SPLICING IN CRYPTOCOCCOSIS

While existing antifungal agents—including amphotericin B, flucytosine, and triazole derivatives—constitute the current therapeutic arsenal, their clinical utility is frequently constrained by considerable pharmacological drawbacks. These include dose-limiting toxicities, restricted CNS penetration, the drug-resistant fungal strains emergence, and logistical limitations in drug availability and delivery—especially in resource-poor settings [31]. Collectively, these factors underscore the pressing need for innovative, mechanism-driven antifungal interventions with improved selectivity, safety, and global accessibility [36].

2.4.1 Challenges Associated with Existing Antifungal Therapies in Cryptococcosis

a. **Toxicity and Limited Therapeutic Window**: Potent antifungals such as amphotericin B are frequently associated with substantial nephrotoxicity, infusion-related adverse reactions, and electrolyte imbalances. While lipid-based formulations offer improved safety profiles, their high-cost limits widespread use in endemic, resource-limited settings. Flucytosine, typically administered in combination regimens, is also constrained by hematologic and hepatic toxicities. Azole antifungals, though generally better tolerated, are not without risk—particularly hepatotoxicity, cardiac arrhythmias (e.g., QT prolongation), and extensive cytochrome P450-mediated drug interactions [31].
b. **Inadequate Central Nervous System Penetration**: Effective management of cryptococcal meningoencephalitis requires antifungals with sufficient cerebrospinal fluid (CSF) penetration. However, the pharmacokinetic properties of many agents fail to ensure consistent CNS bioavailability, often necessitating higher systemic doses that further increase the risk of toxicity without guaranteeing therapeutic success [31].
c. **Fungistatic Nature of Azoles**: Azoles, including fluconazole, generally exert fungistatic rather than fungicidal activity against *Cryptococcus* spp. This limitation is especially problematic in immunocompromised patients who cannot effectively clear the pathogen, thereby increasing the risk of persistent infection and disease relapse.
d. **Emerging Antifungal Resistance**: Prolonged or suboptimal antifungal exposure, particularly fluconazole monotherapy, has been associated with the emergence of *Cryptococcus* strains which are antimycotic resistant. Resistance mechanisms include overexpression of efflux transporters, chromosomal anomalies, and mutations in target genes such as *ERG11*, thereby compromising treatment efficacy and reducing therapeutic options.
e. **Limited Fungal-Specific Targets**: The eukaryotic nature of fungi results in significant molecular homology with host cells, limiting the availability of pathogen-specific drug targets. Consequently, antifungal agents often exert off-target effects in human cells—for instance, interference with cholesterol pathways during ergosterol biosynthesis inhibition—thereby contributing to host toxicity.

f. **Economic and Operational Barriers**: The financial burden of long-term antifungal therapy, hospitalization requirements for intravenous treatments (e.g., amphotericin B), and the need for advanced diagnostic infrastructure pose significant challenges, especially in low-income countries where cryptococcosis burden is highest.

2.4.2 Rationale for Targeting Intein Splicing: A Fungal-Specific, Host-Sparing Therapeutic Paradigm

The persistent shortcomings of existing antifungal pharmacotherapies necessitate a transformative shift toward precision-targeted, low-toxicity interventions that exploit mechanistically distinct fungal vulnerabilities [41]. Among the most compelling of these is the intein-driven protein splicing pathway—a post-translationally done autocatalytic process integral to the viability of numerous fungal pathogens, including *Cryptococcus neoformans*, yet strikingly absent from the human proteome. This unique phylogenetic exclusivity renders intein splicing an exceptionally attractive and untapped therapeutic target [34].

2.4.3 Inteins: Molecular Characteristics and Therapeutic Relevance

Inteins, or intervening protein sequences, are autocatalytic protein segments that mediate a unique post-translational splicing event, wherein they excise themselves from within precursor polypeptides and catalyse the precise ligation of adjacent extein regions to generate a mature, bioactive protein [43]. This intricate process unfolds through a redox-sensitive, metal-ion-responsive cascade of nucleophilic substitutions, including an initial N-terminal acyl shift, transesterification, and a C-terminal asparagine cyclization—executed without the aid of external enzymes or cofactors [35].

In *Cryptococcus neoformans*, a prominent example of intein integration is found within the Prp8 or pre-mRNA processing factor 8, a conserved and indispensable component of the spliceosome complex. Inhibition of intein excision from Prp8 leads to the intracellular accumulation of misprocessed protein precursors, collapse of spliceosomal fidelity, and interruption of RNA maturation [33]. This targeted disruption proves fatal to the fungal cell, positioning Prp8 intein splicing as a highly selective and mechanistically novel target for antifungal drug development.

2.4.4 Strategic Advantages of Intein-Directed Antifungal Intervention

a. **Exceptional Selectivity and Host Cell Preservation:** Intein splicing is an evolutionary feature uniquely absent from metazoan systems, including the human proteome. As such, pharmacological inhibition of this pathway offers unparalleled specificity, effectively circumventing the host toxicity issues frequently encountered with agents targeting conserved eukaryotic pathways such as ergosterol biosynthesis or nucleic acid metabolism [37].
b. **Broad-Spectrum Potential Across Diverse Pathogens:** The evolutionary conservation of inteins across multiple fungal pathogens and select bacterial species presents a unique opportunity for the development of broad-spectrum antifungal therapeutics. Unlike ergosterol-targeted approaches, whose relevance can be species-specific, intein splicing is typically essential for the development and maturation of critical proteins across beings where it exists.

c. **Mechanistic Novelty with Reduced Resistance Risk:** Intein splicing proceeds via a distinct autocatalytic mechanism involving highly conserved nucleophilic residues, notably cysteine and asparagine. This orthogonality to existing antifungal targets significantly diminishes the potential for cross-resistance and offers a novel mechanistic avenue for antifungal drug development, particularly in the face of rising resistance to traditional therapies [35].
d. **Compatibility with Phytochemical Scaffolds and Rational Inhibitor Design:** The pharmacological tractability of intein inhibition has been demonstrated by synthetic molecules such as 6G-318S. Additionally, several naturally existing phytochemicals—few of them being curcumin, EGCG or epigallocatechin gallate, and nimbin—exhibit redox-altering or metal-chelating properties capable of disrupting intein catalysis. These compounds serve as promising low-toxicity scaffolds for rational drug design and structure-based optimization [37].

2.5 Prp8 INTEIN IN *Cryptococcus neoformans*

The Prp8 or pre-mRNA processing factor 8 constitutes the largest and most evolutionarily preserved catalytic component of the eukaryotic spliceosomal RNA-protein complex, essential for coordinating precise intron removal and exon ligation through its interactions with small nuclear RNAs (snRNAs), pre-mRNA substrates, and spliceosomal cofactors. In *Cryptococcus neoformans*, a neurotropic basidiomycete responsible for fatal meningoencephalitis, Prp8 uniquely harbours a mini-intein—a self-splicing protein element that lacks the homing endonuclease domain yet retains the critical catalytic core necessary for post-translational excision. This intein's excision is indispensable for producing the mature, functional Prp8 protein, integral to spliceosomal integrity and RNA processing fidelity. Its compact structure and essential placement within a vital gene underscore both its biological significance and vulnerability to targeted inhibition [33][39]. Notably, inteins are absent in the human proteome, conferring an exceptional degree of selectivity for antifungal intervention. Consequently, pharmacological blockade of the Prp8 mini-intein splicing offers a novel, highly specific therapeutic avenue to disrupt fungal mRNA maturation and viability without detrimental effects on host cells.

2.5.1 Structural and Functional Significance of the Prp8 Intein

In *Cryptococcus neoformans*, the intein sequence embedded within the Prp8 protein represents a catalytically autonomous polypeptide segment strategically inserted into a highly conserved and functionally essential domain of the Prp8 gene. This intein is not a passive genetic artifact, but a critical post-translational regulatory element whose precise excision is indispensable for the correct folding, conformational integrity, and functional activation of the Prp8 protein. Incomplete or failed splicing results in the accumulation of non-functional Prp8 precursors, incapable of assembling into the spliceosomal core [39]. Consequently, the fidelity of pre-mRNA processing is compromised, halting transcript maturation and disrupting downstream gene expression—a vulnerability that highlights the intein as a viable antifungal target.

The Prp8 intein in *Cryptococcus neoformans* undergoes a highly orchestrated four-step canonical protein splicing reaction, beginning with an N–S (or N–O) acyl shift at the N-terminal splice junction, followed by a transesterification reaction with the first residue of the C-terminal extein [4]. This is succeeded by cyclization of a conserved terminal asparagine,

culminating in intein release and the spontaneous formation of a native peptide bond that seamlessly joins the flanking exteins [4][5]. This autocatalytic process is mediated by a conserved constellation of nucleophilic residues—typically cysteine, serine, threonine, and asparagine—embedded within a structurally defined splicing motif [39]. The precise spatial orientation and chemical reactivity of these residues are essential not only for intein excision but also for the accurate restoration of Prp8's tertiary architecture [4]. Successful splicing is thus critical for reconstituting the mature, catalytically competent Prp8 protein, which is indispensable for proper spliceosome assembly and pre-mRNA processing. Disruption of this splicing cascade compromises RNA maturation and presents a strategically vulnerable node for antifungal intervention [4].

2.5.2 Prp8 Intein as a Strategically Druggable Node in Cryptococcosis

The Prp8 intein presents a compelling antifungal target due to its distinct phylogenetic confinement. While the Prp8 protein itself is evolutionarily conserved across all eukaryotic lineages, the presence of an intein within this essential spliceosomal component is restricted to certain microbial pathogens, including numerous fungal species and some archaea [40]. Unlike its fungal counterpart, the human analogue of Prp8 gene i.e. PRPF8 gene lacks intein insertions, producing a complete, functional polypeptide that does not require splicing after the translation is completed. This pivotal evolutionary difference creates a highly specific therapeutic window—allowing selective targeting of the intein present in the Prp8 protein of *Cryptococcus neoformans*. Inhibiting this fungal-specific splicing process can disrupt critical steps in RNA processing and protein maturation within the pathogen, while leaving human cellular machinery unaffected, thereby minimizing host toxicity.

The Prp8 protein in *C. neoformans* contains an intein sequence that must be precisely excised for the protein to become fully functional and integrate into the spliceosomal complex. This requirement positions the intein as a vital regulatory element and a druggable vulnerability. Interference with intein splicing halts the maturation of Prp8, impairing mRNA splicing and reducing fungal viability. Small molecules such as 6G-318S exploit this vulnerability by binding to reactive cysteine residues in the intein's active site. Their mechanisms may include oxidative modification, transition metal chelation, or covalent binding, all of which converge to block intein excision and thereby suppress the growth and survival of the fungal pathogen with high specificity.

2.6 PHYTOCHEMICALS AND THEIR POTENTIAL THERAPEUTIC BENEFITS IN CRYPTOCOCCOSIS

The escalating incidence of antifungal resistance coupled with the intrinsic limitations of existing pharmacotherapies necessitates the exploration of innovative and highly selective therapeutic strategies against cryptococcosis. In this regard, phytochemicals—bioactive secondary metabolites extracted from medicinal plants—have garnered substantial interest owing to their remarkable chemical diversity, multi-target mechanisms, and superior safety profiles relative to synthetic antifungal agents [54]. These naturally derived compounds typically exhibit lower systemic toxicity and can be optimized through precise dosage modulation, advanced delivery systems, and synergistic combinations with other antifungal drugs, enhancing both efficacy and tolerability.

In the specific context of *Cryptococcus neoformans*, a neurotropic fungal pathogen responsible for life-threatening meningoencephalitis, phytochemicals present a novel and promising approach by targeting a uniquely fungal post-translational process: intein-mediated protein splicing within the essential Prp8 protein [56]. The Prp8 intein is a self-excising polypeptide insertion that must be precisely removed to produce a functional spliceosomal protein critical for mRNA processing. This mechanism is evolutionarily absent in human cells, which lack inteins in their homologous PRPF8 gene, thereby conferring a remarkable degree of pathogen-specific vulnerability and minimizing potential off-target effects on the host.

This investigation advances a pioneering antifungal paradigm cantered on the phytochemical-mediated disruption of intein splicing within the Prp8 protein of *C. neoformans*. The evolutionary conservation of inteins across select microbial pathogens and their absolute absence in the human proteome provide a compelling rationale for this approach. Plant-derived bioactives, many of which exhibit redox activity, metal-ion chelation, or electrophilic properties, are well-suited to target the catalytic cysteine and asparagine residues integral to the intein's self-splicing activity. Moreover, these compounds offer a versatile scaffold for drug development, as demonstrated by in silico docking studies employing known intein splicing inhibitors such as 6G-318S, which validate the feasibility of phytochemical interaction with the intein's catalytic core.

By leveraging the unique biological features of fungal inteins and the pharmacological richness of phytochemicals, this strategy embodies a highly selective, mechanistically refined, and host-sparing antifungal intervention. It holds substantial promise in addressing the urgent need for novel therapeutics capable of circumventing the pervasive issue of antifungal resistance. Furthermore, this approach could facilitate the development of next-generation antifungal agents characterized by improved safety, specificity, and efficacy against cryptococcal infections, thereby significantly enhancing clinical outcomes and patient care.

2.7 OVERVIEW OF 6G-318S (CONTROL)

6G-318S is a distinguished small-molecule inhibitor that selectively targets the intein splicing mechanism—a pivotal post-translational modification indispensable for the activation of certain pathogenic proteins, notably within *Cryptococcus neoformans* and related fungal pathogens. This compound has become a cornerstone in contemporary in silico and in vitro studies, serving as a critical reference inhibitor to validate intein-focused antifungal strategies [59].

At the molecular level, 6G-318S exhibits pronounced affinity for the conserved cysteine-enriched catalytic domain of the intein, essential for initiating the autocatalytic excision process. By binding to this domain, 6G-318S disrupts the initial nucleophilic attack that catalyses the N–S acyl rearrangement, effectively arresting the intein splicing cascade [58][64]. This inhibition results in the accumulation of immature, non-functional precursor polypeptides, thereby abrogating the maturation of vital proteins such as the Prp8 spliceosomal factor and culminating in impaired fungal viability [58].

The mechanistic basis of 6G-318S activity may involve metal ion chelation within the active site or oxidative modification of key thiol residues, culminating in the irreversible inactivation

of intein catalytic function. This targeted interference highlights the compound's exceptional specificity, which minimizes off-target interactions and enhances its therapeutic potential [64].

The characterization of 6G-318S has not only substantiated intein splicing as a viable antifungal target but also provided a foundational scaffold for the rational design of novel inhibitors. Its role as a pharmacological benchmark facilitates the advancement of phytochemical and synthetic derivatives with improved safety profiles and multi-faceted bioactivities, thereby fostering the development of next-generation antifungal agents poised to overcome the limitations of existing therapies [59].

CHAPTER 3

COMPUTATIONAL TOOLS AND DATABASE USED

To facilitate the in-silico evaluation of plant-derived inhibitors targeting the Prp8 intein of *Cryptococcus neoformans*, this study incorporated an integrated computational pipeline supported by a suite of freely accessible software tools and specialized bioinformatics databases. These resources were instrumental in performing structure-based virtual screening, molecular modelling, docking simulations, and pharmacokinetic predictions. The tools and repositories employed include:

a. **PyRx – Virtual Screening and Binding Affinity Prediction**: PyRx (https://pyrx.sourceforge.io/) is an open-source computational platform designed for molecular docking and virtual screening. Built upon the AutoDock Vina engine, it enables the simulation of protein–ligand interactions and prediction of binding affinities by evaluating thermodynamic stability and spatial fit [60]. The PyRx software was instrumental in performing large-scale virtual screening of phytochemicals against the intein segment carrying Prp8 protein. It enabled the selection of promising compounds based on their binding or interaction affinities and favourable conformations within the active site.

b. BIOVIA Discovery Studio Visualizer – Structural Analysis and Interaction Mapping: BIOVIA Discovery Studio Visualizer (https://discover.3ds.com/discovery-studio-visualizer-download) provided a robust platform for molecular modelling, structure optimization, and detailed examination of docked complexes [53]. It allowed visualization of binding interactions in 2D and 3D formats, revealing critical contacts such as hydrogen bonds, van der Waals forces, and ionic interactions. It was used here to validate ligand orientation within the intein's active region.

c. SwissADME –ADME Profiling: SwissADME (http://www.swissadme.ch/), web tools from the Swiss Institute of Bioinformatics, supported pharmacokinetic evaluation. SwissADME assessed drug-likeness by analysing pharmacokinetic parameters, including absorption, metabolism, and distribution potential [61]. These tools provided valuable insights into the pharmacological behaviour, bioavailability, and drug-likeness of the phytochemicals studied, thus aiding in the rational selection of compounds with therapeutic potential and minimal off-target effects [61].

d. **IMPPAT – Database of Indian Medicinal Plants and Phytochemicals**: IMPPAT (https://cb.imsc.res.in/imppat/) is a curated repository cataloguing Indian medicinally valuable plants, their constituent phytocompounds, and documented therapeutic indications [65]. Currently encompassing data on over 4,000 plant species and nearly 18,000 phytoconstituents, IMPPAT served as the principal source for bioactive compound selection in this study [65]. This database bridges ethnopharmacological knowledge with modern computational methodologies, allowing the screening of compounds with traditional therapeutic relevance for novel antifungal applications [65].

e. **PubMed – Biomedical Literature Repository**: PubMed (https://pubmed.ncbi.nlm.nih.gov/) was extensively employed to gather current and historical literature related to fungal pathogenesis, intein biology and their splicing mechanism, antimycotic drug mechanisms, and phytocompound bioactivity [66]. It provided the foundation for a comprehensive literature review, ensuring that the computational approach adopted in this study was both scientifically grounded and aligned with existing research trajectories [66].

f. **PubChem – Chemical Compound Information Hub**: PubChem (https://pubchem.ncbi.nlm.nih.gov/) is the world's largest public repository of chemical information, including compound structures, physicochemical properties, biological activities, toxicity data, and associated literature [67]. It was used to retrieve canonical structural data (such as SMILES and InChI keys) for selected phytochemicals, which were then processed for docking and ADME analysis [67]. PubChem's robust data ensured accurate ligand characterization and enhanced reproducibility in downstream computational experiments [67].

g. **Protein Data Bank (PDB) – Repository of Macromolecular Structures**: The Protein Data Bank (https://www.rcsb.org/) houses experimentally resolved 3D structures of proteins, nucleic acids, and their complexes, obtained via X-ray crystallography, NMR spectroscopy, or cryo-electron microscopy [68]. For this study, PDB was used to retrieve or model structural templates of the Prp8 protein to serve as docking targets. Accurate structural representation of the target protein was essential for evaluating the steric and electrostatic compatibility of phytochemical ligands [68] [69].

CHAPTER 4

RESEARCH DESIGN AND PROTOCOLS

4.1 DATA COLLECTION

The present investigation adopted a systematic and integrative approach to data collection, drawing upon an array of bioinformatics tools and specialized biological databases to support the structure-based drug discovery process. The methodology was designed to ensure both depth and precision in the identification and analysis of potential phytochemical inhibitors targeting the intein domain of the *Cryptococcus neoformans* Prp8 protein.

To establish a robust conceptual framework, *PubMed*, the premier biomedical literature database, was extensively mined for peer-reviewed publications relevant to intein biology, fungal pathogenicity, antifungal resistance mechanisms, and phytochemical therapeutics. An in-depth analysis of earlier research formed the basis for this work, directing the identification of suitable molecular targets and shaping the compound screening methodology [66].

Detailed 3D structural data for the Prp8 protein and associated macromolecules were sourced from the Protein Data Bank (PDB), a well-established repository for experimentally derived protein structures [68][69]. In cases where complete crystallographic information was unavailable—especially around the intein insertion site—homology modeling and computational prediction methods were used to accurately reconstruct the missing regions [69].

For ligand selection, phytochemical compounds were chosen from the IMPPAT database, which compiles information on Indian medicinal plants and their therapeutic constituents [65]. This curated resource provided access to a vast repository of phytochemicals derived from Indian medicinal plants, encompassing molecular structures, known bioactivities, and traditional therapeutic uses. The selection criteria were guided by pharmacological relevance, intein-targeting potential, and documented antifungal properties.

To supplement and validate structural information on the selected ligands, PubChem—the world's largest open-access chemical information database—was utilized [67]. Detailed molecular descriptors including SMILES notations, molecular weights, topological polar surface areas, and canonical identifiers were extracted to support subsequent modeling and docking procedures [67].

All molecular modeling tasks were executed using BIOVIA Discovery Studio, a comprehensive visualization and simulation platform [53]. It was used for ligand and receptor preparation, hydrogen addition, energy minimization, and interaction analysis, allowing high-resolution inspection of docking poses and protein-ligand binding interfaces in both two- and three-dimensional formats [53].

Molecular docking/interaction studies were performed using PyRx, a tool aiding in virtual screening that incorporates the AutoDock Vina algorithm to estimate binding affinities between ligands and the intein-bearing Prp8 receptor [60]. The virtual screening was conducted using PyRx, which streamlined the process of identifying phytochemicals

with strong binding potential and good structural alignment within the active site of the Prp8 intein [45][52].

To further examine the drug potential of these compounds, SwissADME—developed by the Swiss Institute of Bioinformatics—was used to analyze their pharmacokinetic profiles [61]. This tool offered predictions on various ADME (which stands for Absorption, Distribution, Metabolism, and Excretion) parameters, including oral bioavailability, gastrointestinal absorption, and blood-brain barrier permeability. These evaluations were essential in determining how suitable each compound could be for therapeutic development [61].

4.2 PROTEIN STRUCTURE ISOLATION AND ITS PREPARATION

The crystal structure of the *Cryptococcus neoformans* Prp8 intein (PDB ID: 6MX6), resolved at 1.75 Å, was retrieved from the RCSB Protein Data Bank [68] (https://www.rcsb.org/) and used as the target protein for molecular docking. Prior to the docking analysis, the structure was carefully prepared and refined to ensure accurate modeling of ligand–protein interactions.

Initial processing was performed using Biovia Discovery Studio, a widely used platform for molecular editing and visualization [53]. To prevent any potential interference during docking, non-essential components such as water molecules, co-crystallized ligands, and heteroatoms were systematically removed. Although these elements may contribute to the protein's function in vivo, removing them helps narrow the focus to regions of pharmacological interest.

Next, polar hydrogen atoms were added to represent possible hydrogen bond donors and acceptors more accurately, improving the prediction of electrostatic interactions. Non-polar hydrogen atoms were also added to better model hydrophobic regions within the binding pocket. These refinements enhanced the realism of the docking environment. Additionally, Autodock Tools within Discovery Studio was used to assign atomic charges correctly and identify active site residues, ensuring the protein was fully optimized for docking.

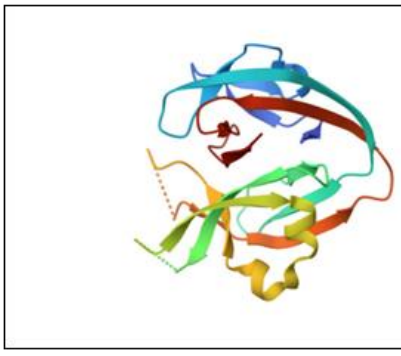
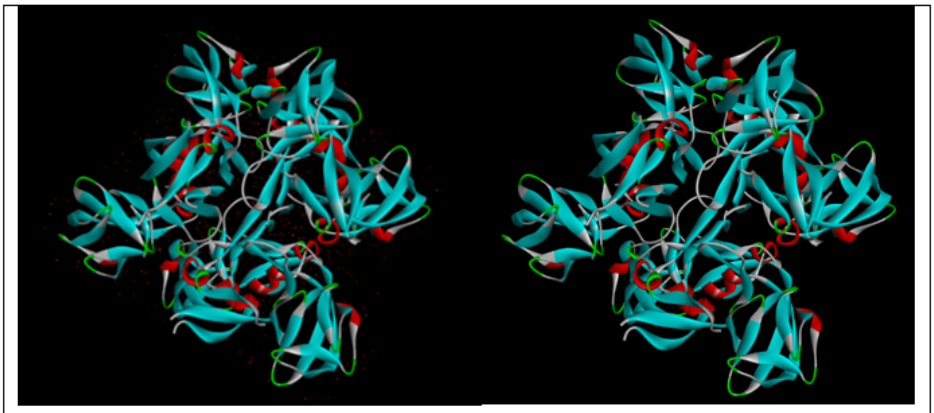


Figure 1. The three-dimensional structure of the ***Prp8 intein protein***, corresponding to PDB ID: **6MX6**, was retrieved from the Protein Data Bank.



A. **B.**

Figure 2. Comparative representation of the Prp8 intein structure (PDB ID: 6MX6) in BIOVIA Discovery Studio. **(A.)** Native form displaying water molecules, heteroatoms, and ligands. **(B.)** Optimized structure post refinement—non-essential elements removed, and polar hydrogens added to enhance structural accuracy for molecular docking.

Accurate determination of partial atomic charges and localization of functional domains were crucial in delineating the Prp8 intein's binding pocket. These sites are known to govern key enzymatic and structural interactions essential for intein splicing, making them prime targets for inhibitor binding [46]. Discovery Studio enabled the precise mapping of these biologically active regions, enhancing the interpretability of docking results and ensuring a rational structure-based drug design approach.

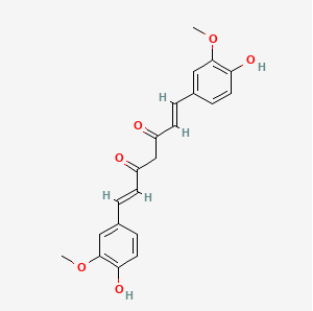
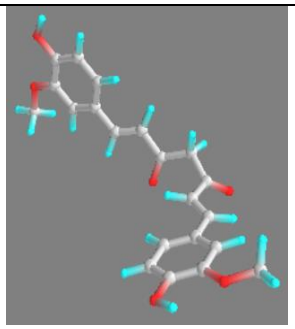
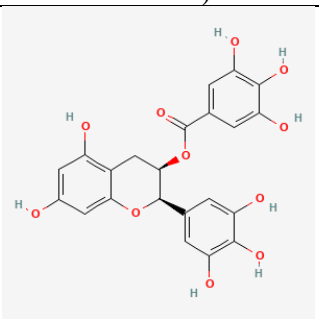
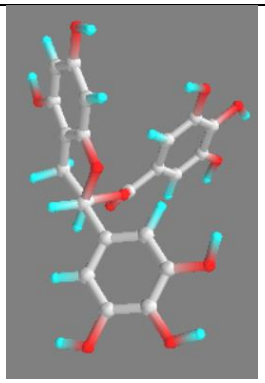
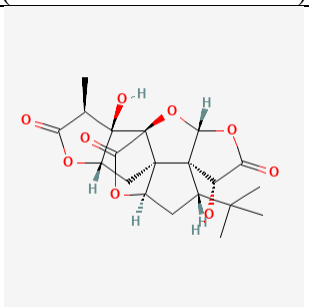
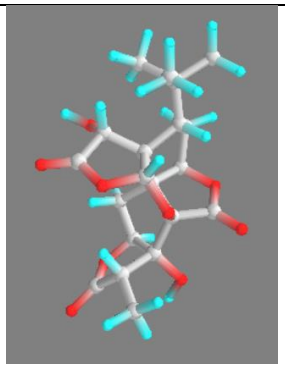
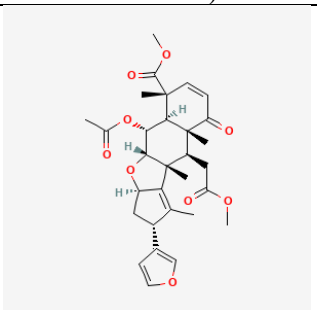
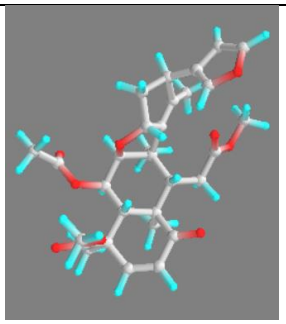
Upon completion of these preparatory steps, the structurally optimized protein model was exported in PDB format, preserving its compatibility with a broad array of docking platforms. This rigorously curated receptor structure was subsequently employed in virtual screening protocols to explore potential interactions with selected phytochemical ligands, thereby laying the foundation for downstream molecular docking and interaction profiling.

4.3 LIGAND ACQUISITION AND PREPARATION OF LIGAND LIBRARY

To explore the inhibitory potential of natural compounds against the *Cryptococcus neoformans* Prp8 intein, five structurally and pharmacologically significant phytochemicals were selected based on their documented bioactivity and relevance in traditional medicine. These included Curcumin, Epigallocatechin gallate (EGCG), Ginkgolide A, Nimbin, and Allicin—compounds derived from *Curcuma longa*, *Camellia sinensis*, *Ginkgo biloba*, *Azadirachta indica*, and *Allium sativum*, respectively. Each of these phytochemicals have been previously demonstrated to exhibit antimicrobial, antioxidant, or anti-inflammatory properties [70], thereby making them promising candidates for antifungal drug discovery [62][70].

The three-dimensional molecular structures of these phytocompounds were retrieved from the PubChem Compound Database (https://pubchem.ncbi.nlm.nih.gov/) in Structure Data File (SDF) format [67]. This format was chosen for its compatibility with molecular

modeling platforms and its ability to retain vital stereochemical, topological, and conformational data necessary for accurate docking [67].

	Phytocompounds	2D Structures and Molecular Formula (Source: PubChem)	3D Structure (Source: PyRx)	Plant Source
I.	**Curcumin**	 $C_{21}H_{20}O_6$ (PubChem CID: 969516)		*Curcuma longa*
II.	**Epigallocatechin gallate (EGCG)**	 $C_{22}H_{18}O_{11}$ (PubChem CID: 65064)		*Camellia sinensis*
III.	**Ginkgolide A**	 $C_{20}H_{24}O_9$ (PubChem CID: 9909368)		*Ginkgo biloba*
IV.	**Nimbin**	 $C_{30}H_{36}O_9$ (PubChem CID:		*Azadirachta indica*

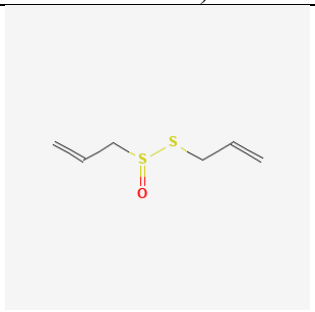
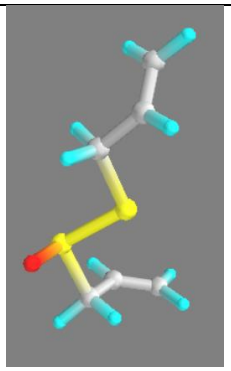
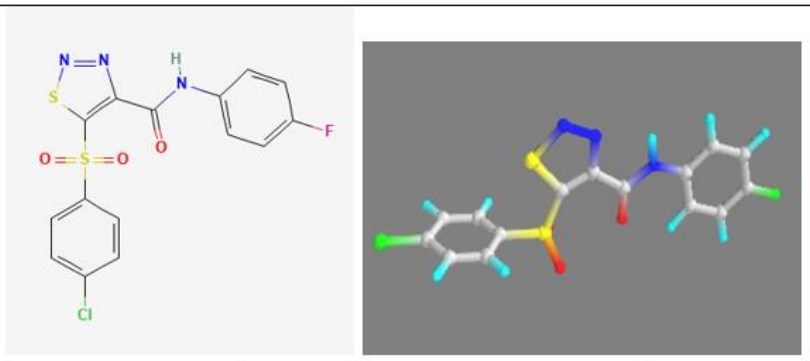
		108058)		
V	**Allicin**	 $C_6H_{10}OS_2$ (PubChem CID: 65036)		*Allium sativum*

Table 2: Representation of the acquired 2D and 3D conformational structures of the selected phytocompounds, illustrating their spatial and planar molecular configurations as retrieved from chemical databases.

In parallel, 6G-318S (PubChem CID: 3788328), a small-molecule compound with established inhibitory activity against intein-containing proteins, was incorporated into the study as a reference or control ligand [51]. Known to interfere with cysteine-mediated nucleophilic catalysis and post-translational splicing events, 6G-318S serves as a mechanistically relevant benchmark for evaluating the comparative binding efficacy of the test phytochemicals. Its 3D conformer was downloaded from the PubChem repository in SDF format.



A. **B.**

Figure 3: **A.** 2D structure of **Control** = 5-[(4-chlorophenyl) sulfonyl]-N-(4-fluorophenyl)-1,2,3-thiadiazole-4-carboxamide [6G-318S (PubChem CID: 3788328)] downloaded from the PubChem Database; **B.** 3D conformer of the control molecule.

Following acquisition, all ligand structures underwent geometry optimization and energy minimization to ensure conformational stability, eliminate steric strain, and achieve a low-energy, biologically relevant pose suitable for docking. This step was critical to reduce conformational artifacts and enhance the reliability of subsequent interaction studies with the Prp8 intein active site.

The final ligand library, comprising the five phytocompounds and the reference inhibitor, was processed and converted into a compatible format for integration into molecular docking workflows, thereby facilitating a high-fidelity evaluation of binding affinities, interaction profiles, and pharmacodynamic potential.

4.4 MOLECULAR DOCKING AND INTERACTION ANALYSIS

To investigate the structural basis of intein inhibition within the *Cryptococcus neoformans* Prp8 protein, a detailed structure-based molecular docking protocol was employed. This approach aimed to simulate and analyse the binding interactions between the intein domain and a set of selected bioactive phytochemicals, alongside a known control compound [45].

The molecular docking was conducted using PyRx (Python Prescription), an open-source virtual screening tool that incorporates AutoDock Vina as its docking engine (https://pyrx.sourceforge.io/) [60]. PyRx supports receptor input in PDB format and ligand input in SDF format, facilitating high throughput in silico screening of small molecules against macromolecular targets.

The 3D crystal structure of the Prp8 intein from *Cryptococcus neoformans* (PDB ID: 6MX6) was retrieved from the RCSB Protein Data Bank (https://www.rcsb.org/). Before docking, the protein underwent refinement using Biovia Discovery Studio Visualizer, a powerful molecular modelling software [53][68][69]. During this step, water molecules, co-crystallized ligands, and non-standard heteroatoms were removed to avoid any potential steric or electrostatic clashes in the docking simulations. To enhance the accuracy of the docking, polar hydrogens were added, and partial atomic charges were assigned to the structure [71].

To establish a comparative control, the small molecule 6G-318S, a known intein inhibitor, was used as the reference ligand [21]. Its 3D conformer was retrieved in SDF format from PubChem (https://pubchem.ncbi.nlm.nih.gov/) [67], minimized for energy optimization, and docked with the prepared active site of the target Prp8 intein [4]. Binding affinity was calculated in kcal/mol, and the output was used to validate the docking protocol.

Following validation, five phytochemicals—Curcumin, Epigallocatechin gallate (EGCG), Ginkgolide A, Nimbin, and Allicin—were selected based on their pharmacological relevance and presence in the IMPPAT database (https://cb.imsc.res.in/imppat/) [65], which catalogues phytochemicals from Indian medicinal plants [65]. These ligands were obtained from PubChem, converted to appropriate formats, and subjected to docking using the same standardized procedure.

Post docking results were investigated through both 2D as well as 3D interaction visualizations using Biovia Discovery Studio, which enabled the identification of key interactions at the molecular scale between the protein and ligands. To further visualize the spatial conformation and validate binding orientation, the docked complexes were loaded into PyMOL (https://pymol.org/2/), a high-resolution molecular visualization system used extensively in structural biology.

The refined receptor-ligand complexes were saved in PDB format for further analysis. The comparative binding energies and interaction profiles indicated that certain

phytocompounds exhibit promising affinity and interaction stability within the Prp8 intein's active site. These compounds potentially engage catalytically relevant residues that may interfere with protein splicing activity—a unique mechanism not commonly targeted by conventional antifungal drugs.

The integrative use of bioinformatics databases and computational docking platforms enabled the identification of novel phytochemical candidates with potential inhibitory effects against the Prp8 intein.

4.5 DOCKING ANALYSIS

In this study, protein-ligand *in-silico* docking and binding studies were employed to investigate the interaction strength and binding profiles of the chosen phytochemicals with our target protein i.e. Prp8 intein retrieved from the genome of *Cryptococcus neoformans* [4]. The overarching objective was to identify compounds exhibiting robust binding energies, potentially equal to or exceeding that of the control compound, 6G-318S—a synthetic molecule known for its effective bioactivity in similar contexts.

The docking simulations were conducted using PyRx, an integrated virtual screening tool that incorporates the AutoDock Vina docking engine (https://pyrx.sourceforge.io/) [60]. Protein structures, formatted in PDB, and ligands, downloaded from PubChem (https://pubchem.ncbi.nlm.nih.gov) in the SDF format, were input into the software for analysis [67]. The docking algorithm calculated the binding affinities of the target protein-ligand associations based on energetically favourable poses within the active site of the Prp8 intein. Binding energies (expressed in kcal/mol) serve as a predictive measure of the strength and stability of the interaction, with lower (more negative) values indicating higher affinity.

To visualize and interpret the binding interactions in detail, the successfully associated (docked) complexes were examined using Biovia Discovery Studio [53], which generated high-resolution 2D and 3D interaction maps. These visualizations illustrated key weak physical interactions and hydrophobic contacts that contribute to ligand stabilization within the binding pocket. An elaborate examination of the molecular orientation and interaction characteristics of the selected compounds in this study revealed significant insights into their ability to inhibit the Prp8 intein splicing mechanism.

Once the in-silico molecular interaction modelling was completed, the five shortlisted phytochemicals underwent a detailed evaluation to determine their potential for therapeutic use. As a first step, the compounds were assessed using Lipinski's Rule of Five — a commonly applied framework for predicting oral bioavailability [61]. This rule considers critical molecular features such as having a molecular weight under 500 Daltons, no more than five hydrogen bond donors, ten or fewer hydrogen bond acceptors, and a LogP value below 5. These characteristics help estimate a compound's ability to be absorbed and utilized within a biological system [72][73][74].

To gain further insights into their pharmacokinetic behaviour, the compounds were subjected to an in silico ADME analysis using SwissADME, an online predictive tool developed by the Swiss Institute of Bioinformatics [61]. This analysis offered predictions on various parameters, including gastrointestinal absorption, blood-brain barrier

permeability, water solubility, and bioavailability score [73]. In addition, the tool checked each compound against other drug-likeness filters, such as those proposed by Veber, Ghose, and Muegge, to assess their alignment with established criteria for orally active drugs [74]. These descriptors are crucial in determining whether a compound is likely to be systemically available and pharmacologically active when administered orally [72].

Collectively, this integrated approach combining molecular docking, pharmacokinetic evaluation, and bioavailability screening allowed for a comprehensive in silico profiling of the selected phytochemicals. The goal was not only to assess binding strength to the Prp8 intein but also to determine whether these compounds possess suitable physicochemical and ADME properties to advance as viable antifungal agents. The findings contribute a significant layer of evidence toward the potential repositioning of plant-derived bioactive compounds as modulators of fungal intein splicing mechanisms—offering new directions for therapeutic intervention against *Cryptococcus neoformans* infections.

CHAPTER 5

RESULTS AND DISCUSSION

5.1 OUTCOMES OF DOCKING FOR THE CONTROL COMPOUND

Ligand-receptor binding/computational docking studies were employed for the examination, evaluation and feasibility of the chosen library of phytochemicals in order to inhibit the intein splicing activity of the Prp8 protein in *Cryptococcus neoformans*. As a benchmark for comparative analysis, the synthetic reference compound 6G-318S, a well-characterized inhibitor of Prp8 intein splicing, was included. It exhibited an interaction affinity of –7.9 kcal/mol, serving as a standard against which the binding performance of the natural compounds was evaluated. Binding affinity values, which reflect the receptor-ligand binding efficiency (i.e. the likelihood of the binding of ligands from the prepared library and the active site of the target protein), are critical indicators of a compound's inhibitory potential—more negative values suggest stronger, more stable and efficient interactions and, by extension, higher likelihood of bioactivity [75].

5.2 DOCKING OUTCOMES FOR PHYTOCHEMICALS

Among the screened phytocompounds, Curcumin demonstrated the most favourable docking outcome, recording a protein-ligand interaction affinity of –8.7 kcal/mol, significantly surpassing the association strength of the control molecule. This indicates a higher binding strength, suggesting that Curcumin may engage the catalytic pocket of the Prp8 intein with enhanced stability. The docking pose revealed key molecular interactions, including several H-bonds and hydrophobic weak contacts, crucial for anchoring the compound within the active site and potentially disrupting the autocatalytic splicing mechanism essential for Prp8 maturation. Inhibition of the splicing in the intein bearing protein and failure in the intein excision may negatively influence spliceosome formation and functionality in the fungal organism. Among the studied compounds, epigallocatechin gallate exhibited the strongest interaction, reflected in a affinity strength of -8.2 kcal/mol. Ginkgolide also showed favourable interaction characteristics, with an energy value of -7.4 kcal/mol. These interactions appear to involve catalytically relevant residues within the intein domain, potentially hindering critical biochemical processes such as nucleophilic cleavage or peptide bond rearrangement required for intein self-excision. The strong interaction profiles of these compounds underscore their capacity to act as competitive inhibitors, possibly impairing intein-mediated activity. Other phytochemicals, such as Nimbin demonstrated interaction affinity of -6.9kcal/mol and Allicin of -4.2kcal/mol. Although these interactions are quite weaker, they still indicate a level of affinity considerably sufficient to suggest biological relevance. These compounds may not fully inhibit the intein but could exert partial or modulatory effects, making them promising secondary candidates for interfering with the intein-mediated maturation process of Prp8.

The results of this study underscore the utility of plant-derived bioactive compounds as promising alternatives or adjuncts to synthetic inhibitors in antifungal drug discovery. The consistent docking behaviour of Curcumin and EGCG—each displaying binding affinities superior to that of 6G-318S—reinforces their candidacy as intein-targeting molecules. Their ability to form energetically favourable and structurally stable complexes with the intein domain may translate into effective inhibition of Prp8 protein processing, thereby compromising the spliceosomal machinery essential for fungal viability. The docking results provide valuable insights into the molecular interactions governing the recognition of ligand and its binding with the target protein. Findings of this research lay strong foundation for subsequent experimental evaluations, including in vitro splicing inhibition assays, fungal growth suppression studies, and lead optimization via structure–activity relationship (SAR) modelling. A ranked summary of all compounds, based on binding affinity, is presented in Table 3, highlighting those with the most promising inhibitory profiles.

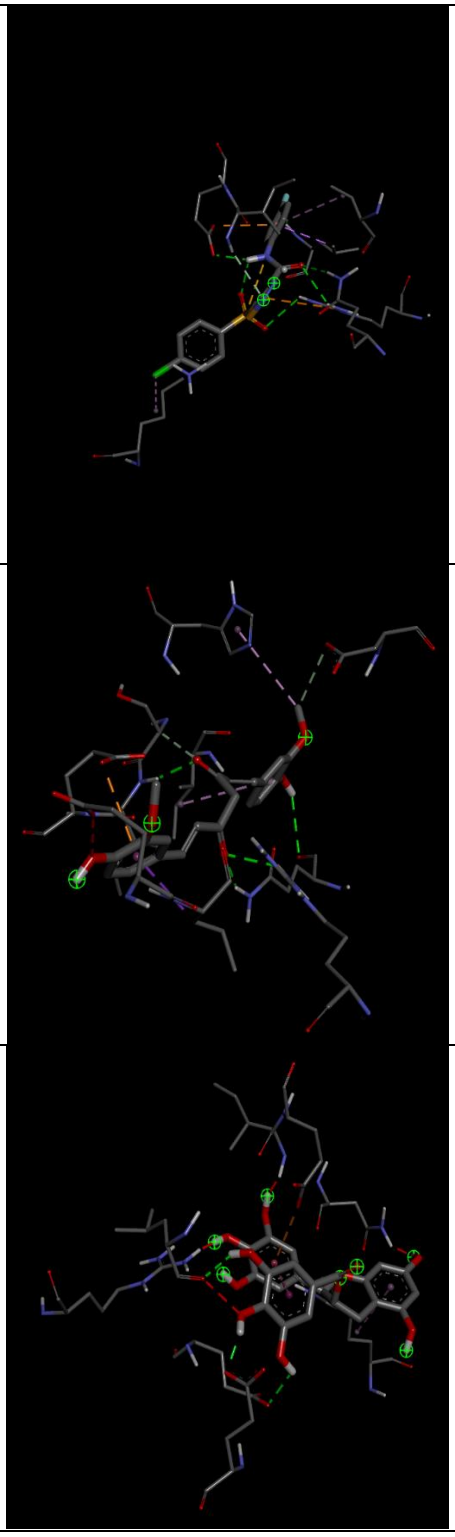
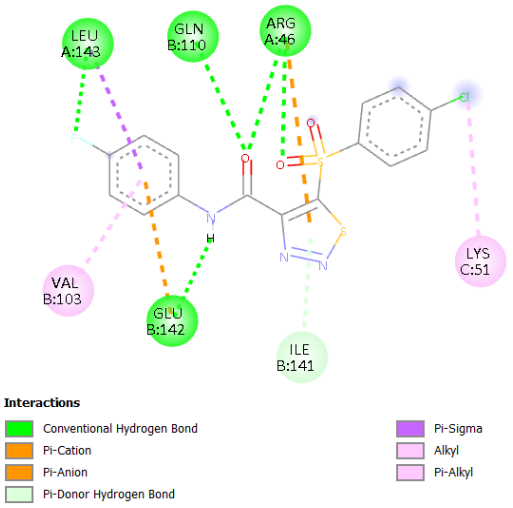
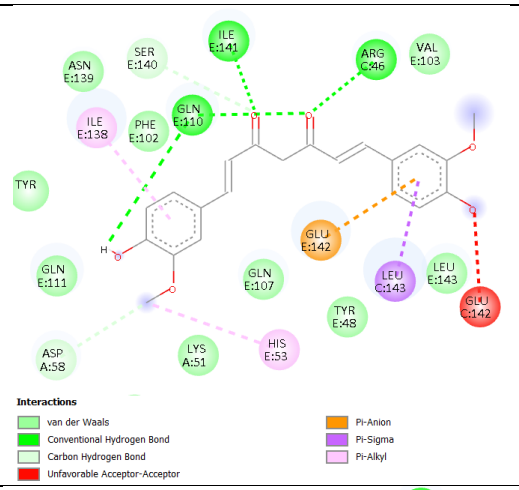
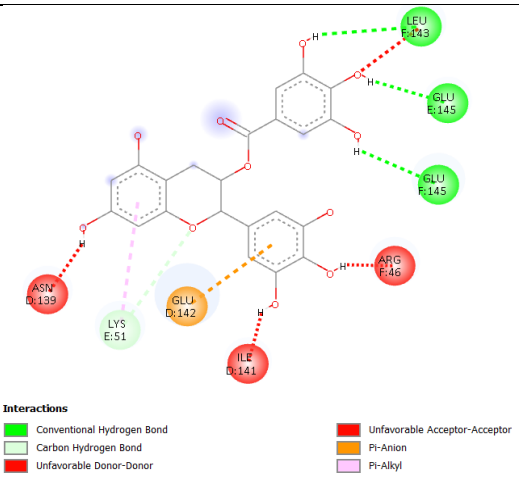
S.No.	Ligands	Docking-based affinity scores (Kcal/mol)
I.	Curcumin	-8.7
II.	Epigallocatechin gallate (EGCG)	-8.2
III.	Gingkolide A	-7.4
IV.	Nimbin	-6.9
V.	Allicin	-4.2
VI.	6G-318S(Control)	-7.9

Table 3: Ligands and their in-silico determined interaction energies.

5.3 VISUAL ANALYSIS OF MOLECULAR CONFORMATION

Following molecular docking, a detailed conformational analysis of the ligand–protein complexes was performed utilizing BIOVIA Discovery Studio to precisely delineate the associations of docked phytochemicals with the protein's active site. Generation of high-fidelity three-dimensional (3D) models elucidated the exact spatial positioning along with molecular interaction conformations of the ligands within the receptor protein's interacting cavity, highlighting their molecular complementarity between their functional groups and specific amino acid residues. Key stabilizing non-covalent interactions—such as hydrogen bonding, hydrophobic contacts, π-π stacking, and van der Waals forces—were thoroughly identified and characterized, providing insight into the structural underpinnings of binding affinity.

Complementary two-dimensional (2D) interaction diagrams distilled this complex information into accessible representations, mapping the critical residues involved in ligand engagement and revealing interaction hotspots responsible for affinity and selectivity. Such in-depth structural interpretations are indispensable for rationalizing ligand efficacy and serve as a foundation for the iterative optimization of these phytochemicals as potent antifungal agents. Ultimately, this integrative analysis advances the strategic development of targeted inhibitors against the Prp8 intein, reinforcing their translational potential in combating Cryptococcus neoformans infections. (Table 4)

Compounds	Visualization in 3-D	Visualization in 2-D
6G-318S (Control)		
Curcumin		
Epigallocatechin gallate (EGCG)		

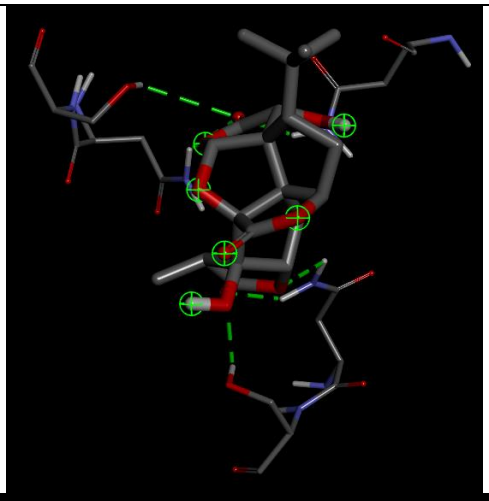
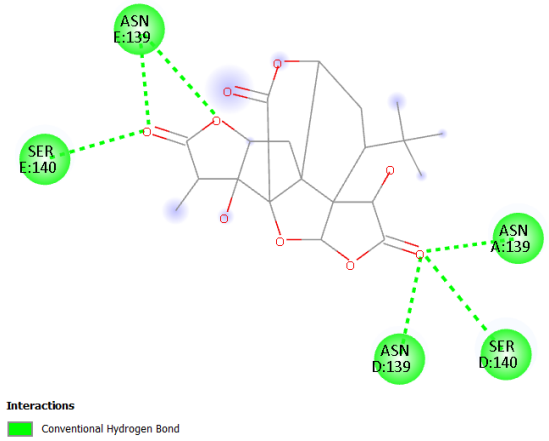
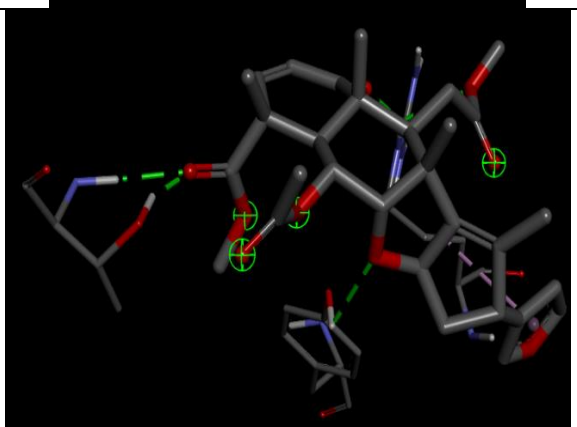
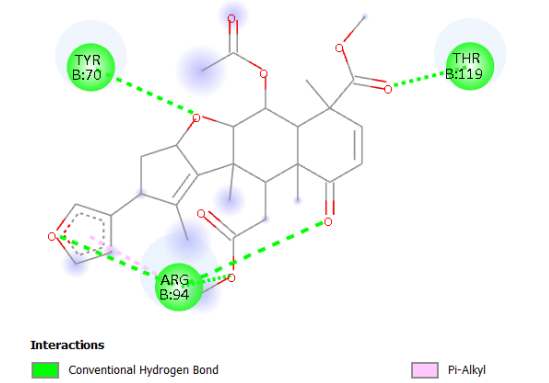
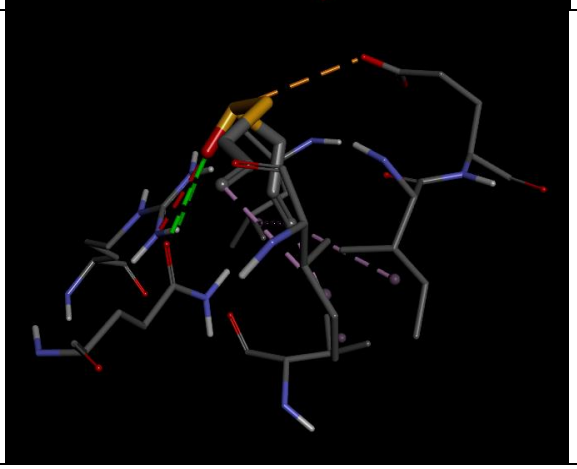
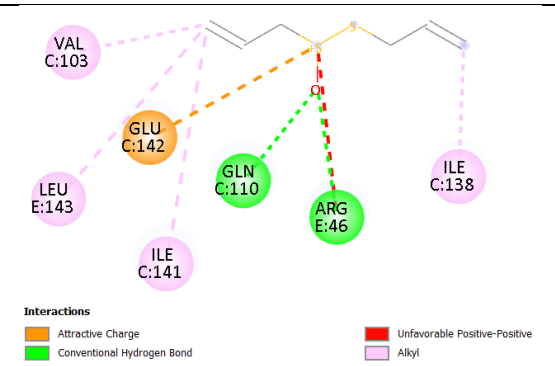
Gingkolide A		
Nimbin		
Allicin		

Table 4: Structural representation of the 3-D binding conformations & corresponding 2-D interaction profiles of selected phytocompounds and control compound with the Prp8 intein protein, highlighting key molecular interactions within the active site.

5.4 ADME EVALUATION

SwissADME is an accessible online platform frequently applied in the preliminary stages of drug design to evaluate how small, bioactive molecules—such as phytocompounds—might perform within the human body [61]. It predicts a variety of pharmacokinetic attributes, including absorption through the digestive tract, chemical stability, lipophilicity, and solubility in aqueous environments [61]. Additionally, it offers insights into whether a compound aligns with

established drug likeness criteria, aiding in the identification of candidates suitable for further pharmaceutical development [61].

Within SwissADME, the BOILED-Egg model serves as a visual predictor of how likely a compound is to be absorbed in the gastrointestinal tract or to reach the brain by crossing the blood–brain barrier (BBB) [61]. In the model's output, compounds that appear in the yellow region are considered likely to penetrate the BBB, while those outside this area are not. This tool enables a quick and intuitive evaluation of a compound's potential for central nervous system involvement [61].

SwissADME also assesses if a compound is likely to be transported by P-glycoprotein (P-gp), a crucial protein that pumps substances out of cells and can influence how drugs are absorbed and distributed [76]. Molecules predicted as P-gp substrates are marked with blue dots (P-gp+), meaning they might be actively removed from areas like the brain or gut, which could reduce their effectiveness [76]. In contrast, compounds labeled with red dots (P-gp–) are not recognized by this transporter, indicating a higher probability of successful absorption and therapeutic action [76].

Molecule (Bioavailabil -ity Score)	MW (g/mo l)	Consens -us Log P	Water Solubilit -y, Log S (ESOL)	Water Solubili -ty (ESOL class)	Absorpti -on in GI	BBB Permiss ible	P-gp	Log Kp (cm/ s)	Lipinsk i Violatio -ns	Leadliken- ess Violations	Synth etic Acces- si-- bility
6G-318S	397.83	3.22	-4.67	Moderate - -ly soluble	Low	No	No	-6.26	0	1	3.13
Curcumin	368.38	3.03	-3.94	Soluble	High	No	No	-6.28	0	2	2.97
Epigallocatech in gallate (EGCG)	458.37	0.95	-3.56	Soluble	Low	No	No	-8.27	2	1	4.20
Ginkgolide A	408.40	-0.60	-2.68	Soluble	High	No	Yes	-8.37	0	1	6.28
Nimbin	540.60	3.17	-4.20	Moderate ly soluble	High	No	No	-7.98	1	2	6.54
Allicin	162.27	1.61	-1.34	Very Soluble	High	Yes	No	-6.36	0	1	3.60

Table 5: SwissADME data for five phytocompounds and reference molecule 6G-318S, covering essential pharmacokinetic and drug-likeness features such as absorption, BBB permeability, P-gp substrate status, and drug-likeness rules to evaluate their potential as oral drug.

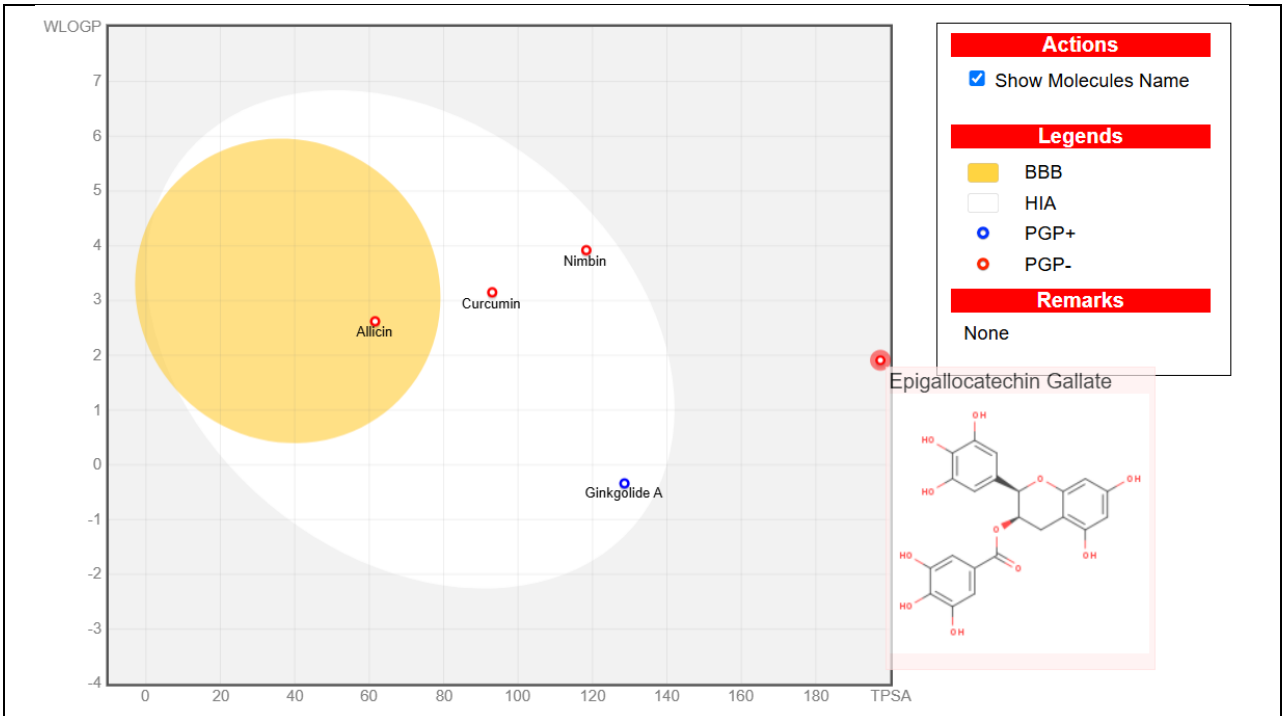


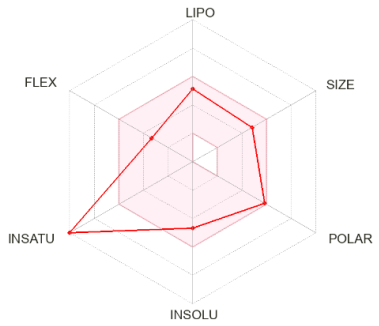
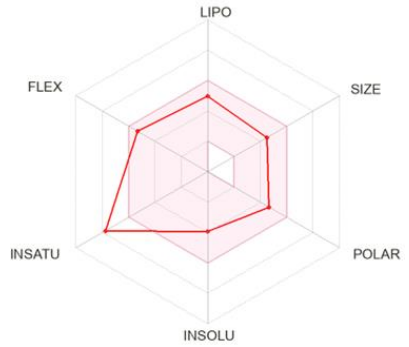
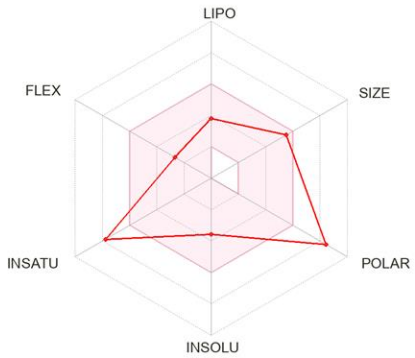
Figure 4: BOILED Egg illustration of the chosen bioactive compound library
Curcumin, Allicin, and Nimbin are predicted to have strong gastrointestinal absorption. Allicin also shows the ability to cross the blood–brain barrier. Ginkgolide A and EGCG are less likely to be absorbed or reach the brain, with Ginkgolide A identified as a P-glycoprotein substrate that may limit its accumulation. Among these, Allicin is the most promising for both oral absorption and brain access.

In addition to the BOILED-Egg, SwissADME also generates radar plots to visually summarize a compound's drug-likeness based on multiple molecular descriptors, aiding in the rational prioritization of lead compounds for further experimental validation [76][61].

The bioavailability radar plot, accessible via the SwissADME online tool (http://www.swissadme.ch/), provides an integrative and highly visual representation of a compound's drug-likeness by simultaneously assessing six pivotal physicochemical properties [76][61]. These properties include lipophilicity (which influences membrane permeability), molecular size (affecting absorption and distribution), polarity (impacting solubility and interaction with biological targets), solubility (crucial for bioavailability), flexibility (relating to molecular conformational adaptability), and saturation (reflecting the degree of sp^3-hybridized carbons, important for metabolic stability). Each of these parameters is plotted on a separate radial axis, forming a polygonal shape that succinctly encapsulates the compound's overall ADME (absorption, distribution, metabolism, and excretion) profile.

This multidimensional plot facilitates a rapid and intuitive evaluation of whether the compound's physicochemical attributes fall within the desired ranges for oral bioavailability. Notably, the pink shaded region on the radar plot highlights the optimal parameter windows that are typically associated with favorable pharmacokinetic behavior and drug-like properties. Compounds whose radar polygons reside predominantly within this pink zone are predicted to exhibit efficient gastrointestinal absorption, metabolic stability, and appropriate solubility, making them

promising candidates for further drug development. Consequently, this visualization serves as a powerful early stage screening tool, enabling researchers to efficiently prioritize molecules with balanced ADME profiles, thus accelerating the rational design and optimization of new therapeutics.

Compounds	Bioavailability	Radar Plot Diagrams
6G-318S (Control)	0.55	
Curcumin	0.55	
Epigallocatechin gallate (EGCG)	0.17	

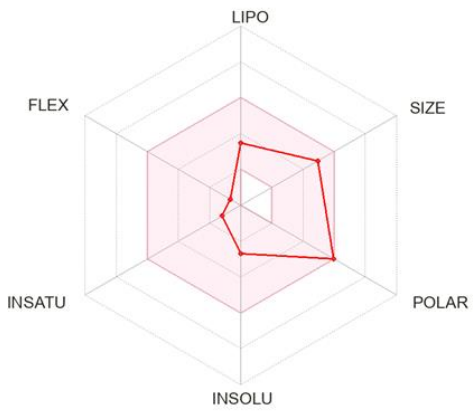
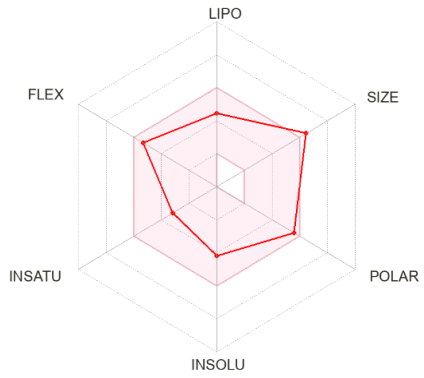
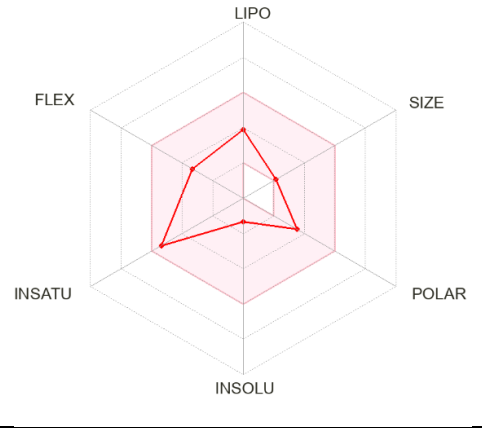
Ginkgolide A	0.55	
Nimbin	0.55	
Allicin	0.55	

Table 6: Summary of the predicted oral bioavailability characteristics of the selected phytochemicals, accompanied by their respective bioavailability radar plots illustrating physicochemical and drug-likeness parameters.

CHAPTER 6

CONCLUSION

Amidst the growing global health concern posed by rising antifungal resistance and the stagnation of novel antifungal drug development, this dissertation presents a targeted and evolutionarily selective therapeutic strategy directed against *Cryptococcus neoformans*. The core of this approach hinges upon the inhibition of intein-mediated splicing within the Prp8 protein—an essential catalytic component of the spliceosome machinery, required for RNA maturation and gene expression in fungal systems. In contrast to canonical antifungal targets, inteins represent a highly promising class of molecular intervention points owing to their catalytic indispensability, pathogen-specific presence, and complete absence in the human host, thereby offering unparalleled therapeutic selectivity and reduced toxicity risk.

This work applied structure-based virtual screening to evaluate five plant-based compounds for their ability to inhibit the intein domain within the Prp8 precursor protein. Docking studies using PyRx and AutoDock Vina assessed binding strengths and interaction details, comparing them to the synthetic inhibitor 6G-318S. Ligand and protein structures were prepared and optimized in Biovia Discovery Studio, with 3D interaction analyses carried out in PyMol and Discovery Studio.

The docking results revealed compelling evidence of strong ligand-receptor interaction, with several phytocompounds demonstrating higher binding affinity than the control molecule. Notably, Curcumin exhibited the most significant interaction energy at –8.7 kcal/mol, outperforming 6G-318S (–7.9 kcal/mol) and indicating a more stable and potentially efficacious inhibitory effect on intein splicing. Epigallocatechin gallate (EGCG) and Ginkgolide also demonstrated robust affinity strengths of –8.2 kcal/mol, -7.4 kcal/mol, reinforcing their candidacy as potent antifungal agents. These interactions were further characterized by favourable hydrogen bonding networks, van der Waals contacts, and hydrophobic surface complementarity within the intein active site, all of which are essential determinants of molecular recognition and binding specificity.

The therapeutic potential of the selected phytochemicals was further examined through SwissADME to analyse their physicochemical and pharmacokinetic traits. Several compounds complied with Lipinski's Rule of Five, meeting key drug-likeness parameters like molecular weight, lipophilicity, hydrogen bonding, and oral bioavailability. The ADME assessment also indicated favourable absorption and metabolic stability, supporting their potential as drug candidates.

These findings not only highlight the structural compatibility and functional efficacy of plant-derived inhibitors but also establish a promising framework for the rational design of intein-targeted antifungal agents. The selective disruption of fungal-specific post-translational processes represents a mechanistically novel and host-sparing therapeutic axis. Moreover, by

leveraging the rich chemical diversity of natural compounds, this approach enhances the pharmacological toolkit for combating cryptococcosis and potentially other fungal pathogens harbouring inteins.

The promising outcomes of this computational study provide a strong rationale for proceeding to experimental stages, including biochemical validation through in vitro intein splicing inhibition assays, fungal culture-based growth inhibition analyses, and in vivo evaluation in cryptococcal infection models.

In essence, this dissertation lays the conceptual and computational foundation for an innovative class of antifungal therapeutics that target intein-bearing proteins. By merging evolutionary selectivity, molecular accuracy, and strong pharmacological potential, this strategy creates a promising path to develop antifungal drugs that can overcome resistance while reducing harm to the host. The integration of phytochemical scaffolds with contemporary drug discovery paradigms could thereby usher in a new era of selective, sustainable, and effective antifungal interventions.

CHAPTER 7

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DECLARATION BY THE CANDIDATE

I, **ANJALI TIWARI** (Roll Number: **2K23/MSCBIO/08**), hereby certify that the dissertation entitled **"Structure-based In-silico Identification of Plant-derived Inhibitors Targeting Prp8 Intein Splicing in *Cryptococcus neoformans*: A Phytochemical Derived Remedy for Antimycotic Drug Resistance"**, is the outcome of my own original research. It is submitted in partial fulfilment of the requirements for the Master of Science in the Department of Biotechnology at Delhi Technological University. This work was carried out independently under the guidance of **Dr. Navneeta Bharadvaja** between May 2024 and May 2025.

To the best of my knowledge, the content incorporated in this thesis has not been submitted, in part or in full, for the award of any other degree or diploma at this or any other institution or university.

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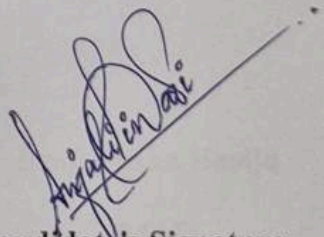

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This is to certify that **Ms. ANJALI TIWARI (Roll No. 2K23/MSCBIO/08)** has undertaken and successfully completed the research work embodied in the present dissertation entitled **"Structure-based In-silico Identification of Plant-derived Inhibitors Targeting Prp8 Intein Splicing in *Cryptococcus neoformans*: A Phytochemical Strategy to Overcome Antifungal Drug Resistance,"** submitted in partial fulfilment of the requirements for the award of the degree of **Master of Science** in the Department of Biotechnology, Delhi Technological University, Delhi.

The work presented in this thesis is the outcome of original and independent research conducted by the candidate under my supervision. To the best of my knowledge, the content of this dissertation has not been submitted, either in part or in full, for the award of any other degree or diploma at this or any other University/Institution.

Place: Delhi
Date: 05.06.2025

Dr. Navneeta Bharadvaja
Associate Professor
Supervisor
Dept. of Biotechnology
Delhi Technological University

Prof. Yasha Hasija

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