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Original Article

Evaluation of *Cordyceps militaris* as a Potential TGF Beta Inhibiting Herbal Therapeutic Agent for Oral Submucous Fibrosis

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ABSTRACT

Background: Oral Submucous Fibrosis (OSMF) is a long-standing, potentially malignant disorder marked by advancing fibrosis of the mucosa in the oral cavity, driven by unregulated Transforming Growth Factor-beta (TGF-β) signaling. Existing treatments are mainly symptomatic and do not reverse fibrosis, necessitating exploration of novel therapeutic agents. Bioactive compounds from *Cordyceps militaris* have shown antifibrotic potential through inhibition of TGF-β pathway. The aim of the study was to evaluate molecular interactions of selected *Cordyceps militaris* compounds with TGF-β type I (TβRI) and type II (TβRII) receptors using *in silico* docking, and assess their drug-likeness and pharmacokinetic properties for treatment of OSMF. **Materials and Methods:** Five compounds-Cordycepin, Adenosine, γ-Aminobutyric Acid (GABA), Ergothioneine, D-mannitol-were retrieved from ZINC15. TβRI (3TZM), TβRII (1M9Z) structures were obtained from the Protein Data Bank. ADME properties were evaluated using SwissADME, molecular docking was performed using BIOVIA Discovery Studio. **Results:** Cordycepin and Adenosine showed strongest binding affinities (-6.4 to -7.1 kcal/mol), forming stable interactions with active site residues. Ergothioneine showed moderate binding, while GABA and D-mannitol showed weaker interactions. Most compounds satisfied Lipinski's rule. **Conclusion:** Cordycepin and Adenosine showed favorable pharmacokinetics. *In silico* findings suggested Cordycepin and Adenosine exhibited promising antifibrotic potential against TGF-β receptors in OSMF, warranting further experimental validation.

Keywords: *Cordyceps militaris*, Drug, Health, Neglected Disease, Oral Submucous Fibrosis, TGF-β.

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INTRODUCTION

Oral Submucous Fibrosis (OSF) is a long-standing debilitating condition affecting the oral mucosa with significant malignant transformation potential (Aarthi *et al*., 2025). Its characteristic features include persistent inflammatory reactions, excessive collagen turnover in the connective tissues below the buccal mucosa, localised inflammatory response in the lamina propria or deeper connective tissue stroma and muscle degeneration (Shih *et al*., 2019). The most frequent site of occurrence is the buccal mucosa. The major presenting symptoms are burning mouth sensation and trismus (Pandiar *et al*., 2024), with a reported malignant transformation rate of 2.3-7.6% (Ali *et al*., 2014). Among potentially malignant disorders of the oral cavity, OSMF was observed to have significant malignant transformation potential, with a likelihood of progression into oral squamous cell carcinoma despite cessation of the confounding etiology factors (Kumar *et al*., 2024). This underscored the rapidly progressive and aggressive biological behavior of the disease and the need for early diagnosis and timely therapeutic intervention. Moreover, the irreversible nature of fibrosis and progressive epithelial atrophy further increased the risk of malignant transformation, thereby making effective treatment strategies a clinical priority (Kumar *et al*., 2024). Despite its high disease burden, current treatment modalities remain primarily symptomatic with the inability to effectively reverse fibrosis or arrest disease progression.

The quality of life of patients with OSMF is severely compromised, as they often experience difficulty in eating, speaking, and performing basic oral functions due to restricted mouth opening (Aman *et al*., 2023). Moreover, there is currently no definitive and effective treatment available that significantly alleviated the symptoms or halt disease progression, Therefore, therapeutic approaches that can target both fibrosis and disease progression are of utmost importance (Chhabra *et al*., 2023) as delayed or inadequate treatment may increase the likelihood of progression to malignancy.

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Etiological risk factors contributing to OSMF include autoimmune disorders, nutritional deficiencies (Vitamins B, C, Iron), enduring pattern of chewing areca nut/paan, spices, Human Papilloma Virus (HPV) infection, and gene alterations (Balakrishnan *et al.*, 2015; Arakeri *et al.*, 2017; Guruprasad *et al.*, 2014; Wang *et al.*, 2015; Teh *et al.*, 2008). These confounding factors contributed to persevering inflammation and uncontrolled wound healing, eventually leading to advancing fibrosis.

Numerous regulatory factors and interleukins play a role in the complex pathogenesis of OSMF, with Transforming Growth Factor Beta (TGF-β) being extremely significant due to its central role in proliferative cell growth and apoptotic cell death of epithelial cells in the oral mucosa. Among variable isoforms (TGF-β1, β2, β3), TGF-β1 and TGF-β2 were observed to be crucial in the onset of Oral Submucous Fibrosis (OSMF). These isoforms mainly signal through TGF-β type I (TβRI) and type II (TβRII) receptors, moderating the downregulation of fibrotic responses. Therefore, targeting the TGF-β pathway may be considered as an effective remedial strategy for OSMF due to its central role in the fibrotic process. TGF-β is a key transcriptional regulator of fibrosis (Aarthi *et al.*, 2025), promoting activation of fibroblasts, overproduction of collagen, and accumulation of Extracellular Matrix (ECM), which are all significant indicators of OSMF progression (Shi *et al.*, 2022). Suppression of TGF-β signaling has been shown to mitigate fibroblast proliferation, decrease collagen deposition, and attenuate the chronic inflammatory response in fibrotic diseases (Kim *et al.*, 2018; Shul *et al.*, 1992; Kulkarni *et al.*, 1993). In addition, blocking TGF-β signaling is useful in restoring normal epithelial-mesenchymal balance and preventing epithelial atrophy, which is commonly observed in OSMF (Tang *et al.*, 2025). Thus, inhibiting TGF-β not only addresses the fibrotic component but also the inflammatory and degenerative aspects of the disease, making it a holistic approach for management of OSMF (Bansal *et al.*, 2023). Targeting TGF-β receptors represents a rational and promising strategy for modulating fibrosis at a molecular level in OSMF.

Cordyceps militaris, is a medicinal fungus widely recognized for its antioxidant, anti-senescent, and therapeutic characteristics (Ashraf *et al.*, 2020) has been reported to modulate the TGF-β signalling pathway (Pan *et al.*, 2013). It contains bioactive compounds, Nucleosides-Cordycepin and Adenosine, and other biologically active substances γ-aminobutyric acid (GABA), Ergothioneine, and D-mannitol (referred to as Cordycepic acid) with several health benefits (Jędrejko *et al.*, 2021). The aim of the present study was to investigate the potential of these bioactive compounds as inhibitors of the TGF-β signalling pathway in the context of Oral Submucous Fibrosis (OSMF). Accordingly, the objective of the present *in silico* study was to evaluate the molecular interactions of selected bioactive compounds from *Cordyceps militaris* with TGF-β type I and type II receptors to assess their potential as TGF-β receptor inhibitors in OSMF. Based on this rationale, it was hypothesized that these bioactive compounds can effectively inhibit TGF-β receptors through molecular interactions.

MATERIALS AND METHODS

Ligand Identification and analysis of drug likeness

Bioactive compounds from *Cordyceps militaris* were selected based on their reported biological activities targeting the TGF-β pathway. The ligands included Cordycepin, Adenosine, GABA, Ergothioneine, and D-Mannitol. Their molecular structures were obtained in SDF format from the ZINC15 database (https://zinc15.docking.org/) to obtain docking-ready ligand conformations. These structures were further cross-validated using the ChEMBL and PubChem databases to ensure structural accuracy and biological relevance. The downloaded molecular structures were converted into mol2 format using OpenBabel software to facilitate docking procedures. The formulated structures of ligands were used for molecular interaction and subsequent docking analysis.

Target Protein Structure Retrieval and Binding Pocket Identification

The three-dimensional crystal structures of TGF-β1 Receptor (TβRI; PDB ID: 3TZM) and Type II TGF-β receptor (TβRII; PDB ID: 1M9Z) were extracted in a PDB format from the Protein Data Bank (http://www.rcsb.org). Formulation of proteins included removal of water molecules and heteroatoms along with adding polar hydrogens. The formulated protein structures were saved in.pdbqt format for ensuing docking analysis and were implemented for molecular interaction analysis.

Molecular Docking and Interaction Analysis

Molecular interaction analysis between the selected bioactive constituents of *C. militaris* and TGF-β Type I Receptor (TβRI) and Type II Receptor (TβRII) was performed with BIOVIA Discovery Studio. The ligand-receptor complexes were analyzed to evaluate binding affinities and understand the strength and stability of ligand-receptor interactions.

Visualization of Molecular Interactions

The ligand-receptor complexes were analyzed to identify key mechanisms, including hydrogen bonding, hydrophobic interactions, and electrostatic interactions. These interactions were evaluated to understand the binding patterns and stability of the ligand-receptor complexes. Molecular linkage, including hydrogen bonds, hydrophobic interactions, and electrostatic interactions between the ligands and the proteins, were visualized using BIOVIA Discovery Studio. Primary interacting amino acid residues and bond distances were analyzed to characterize ligand-receptor interactions.

Drug-Likeness Validation of Docked Compounds and ADME Analysis

The curated elements were assessed for medicinal potential and pharmacological characteristics using the SwissADME online tool. Canonical SMILES of each compound were procured from chemical databases and manually entered into the SwissADME server for further assessment. Significant physicochemical parameters, including molecular weight, Lipophilicity (logP), Topological Polar Surface Area (TPSA), number of hydrogen bond donors and acceptors, and rotatable bonds, were evaluated. Assessment of drug-likeness was done based on Lipinski's rule of five, along with Ghose, Veber, Egan, and Muegge filters. Additionally, bioavailability score and pharmacokinetic properties were assessed to predict oral absorption and overall drug potential of the compounds. The drug-likeness and pharmacokinetic characteristics of the selected bioactive components were correlated with their binding affinities to identify potential lead candidates. Compounds presenting with both favorable interaction profiles and acceptable ADME properties were considered for further analysis.

Statistical Analysis

For the *in silico* docking studies, binding affinities were expressed in kcal/mol. Descriptive data analysis was used to compare the binding energies and ADME properties of the five selected bioactive compounds. No further inferential statistical tests were required for this computational modeling study.

Ethical Considerations

The present study was conducted using *in silico* computational methods without including any human participants, clinical samples, or animal models. Hence, ethical approval from an institutional ethics committee was not considered necessary. This research was carried out in association with standard scientific research practices and guidelines.

RESULTS

Ligand Identification and Drug-Likeness Analysis

The molecular structures of five bioactive compounds developed from *Cordyceps militaris*-Cordycepin, Adenosine, GABA, Ergothioneine, and D-Mannitol-were obtained from the ZINC15 database in SDF format and converted to mol2 format using OpenBabel software. These ligands were analyzed for drug-likeness properties using the SwissADME online tool. Most compounds satisfied the criteria of Lipinski's rule of five, as well as Ghose, Veber, Egan, and Muegge filters, indicating a high potential for drug development. Cordycepin and Adenosine exhibited optimal properties, including favorable logP values (1.63 and 0.55, respectively) and bioavailability scores of 0.55. Ergothioneine showed moderate compliance with drug-likeness rules, while GABA and D-Mannitol displayed limitations such as high hydrogen bond donors or topological polar surface area, leading to slightly reduced predicted bioavailability. These results are elaborated in Tables 1 and 2.

Protein Structure Retrieval and Binding Pocket Identification

The three-dimensional structures of TGF-β Type I Receptor (TβRI) and TGF-β type II Receptor (TβRII) were obtained from the Protein Data Bank (PDB). After cautious selection based on quality and significance, structures were developed for molecular docking by removal of water molecules, adding polar hydrogens, and attributing Kollman charges. Active binding sites were identified based on prior literature and structural analysis, ensuring that relevant binding regions were considered for interaction analysis. Figure 1 shows the 3D structures of TGF-β type I receptor (TβRI) along with TGF-β Type II Receptor (TβRII), while Figure 2 displays the binding pockets chosen for docking.

Molecular Docking and Binding Energy Analysis

Molecular docking and interaction analysis were implemented through BIOVIA Discovery Studio to examine the binding affinities between selected compounds and TGF-β Type I Receptor (TβRI) and TGF-β Type II Receptor (TβRII). Among tested ligands, Adenosine presented with the highest affinity of binding toward TβRI (-7.1 kcal/mol), succeeded by Cordycepin (-6.7 kcal/mol), while Ergothioneine displayed moderate interaction (-6.3 kcal/mol). GABA (-4.0 kcal/mol) and D-Mannitol (-4.9 kcal/mol) exhibited comparatively weaker interactions.

Table 1: SwissADME analysis of *Cordyceps militaris* bioactive compounds. Molecular weight, binding energy (kcal/mol), bioavailability score, and Topological Polar Surface Area (TPSA) of each compound with TGF-β type I Receptor (TβRI).

TGF-β type I receptor (TβRI)				
Name	Molecular weight	Binding energy	Bioavailability	TPSA
Cordycepin	251.24	-6.7	Low	112.33
Adenosine	267.24	-7.1	Low	142.56
GABA	103.12	-4	High	63.32
Ergothionine	229.3	-6.3	Moderate	104.74
D-Mannitol	182.17	-4.9	High	120.53

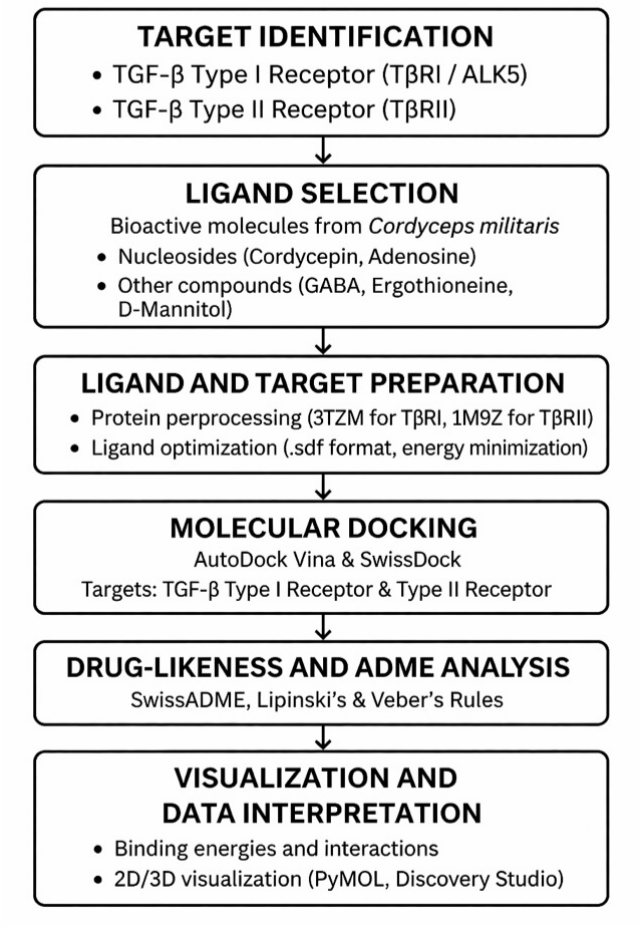


Figure 1: Workflow of *in silico* Molecular Docking Study for *TGF-β* Inhibitor Screening.

Likewise, in TβRII, Adenosine demonstrated the maximum binding affinity (-6.9 kcal/mol), succeeded by Cordycepin (-6.4 kcal/mol), while Ergothioneine presented with moderate binding (-6.3 kcal/mol). GABA (-4.0 kcal/mol) and D-Mannitol (-4.2 kcal/mol) displayed the lowest binding affinities. These results suggested that Adenosine and Cordycepin may act as promising modulators of TGF-β signaling. Comprehensive binding energy values are displayed in Tables 1 and 2 and properties of the selected compounds, including hydrogen bond donors, hydrogen bond acceptors, hydrophobic groups, and charge characteristics are summarized in Table 3.

Visualization of Molecular Interactions

Illustrative molecular docking structures with highest-binding compounds, Cordycepin and Adenosine, interconnecting with TGF-β Type I Receptor (TβRI) and TGF-β Type II Receptor (TβRII) are shown in Figure 3. Cordycepin developed several hydrogen bonds and primary amino acid remnants such as Lys232, Glu245, Asp351, and Ser280 of TβRI, along with stabilizing hydrophobic interactions involving Val219 and Leu340. In TβRII, hydrogen bonding interactions were observed with residues including Lys37, Asp55, Glu119, and Ser80, contributing to stable protein-ligand complex formation. Figure 4 provides 2D interaction diagrams depicting hydrogen bond interactions, hydrophobic contacts, and bond distances. These interaction patterns highlighted the molecular basis of their strong binding affinity and potential therapeutic modulatory effects on TGF-β signaling.

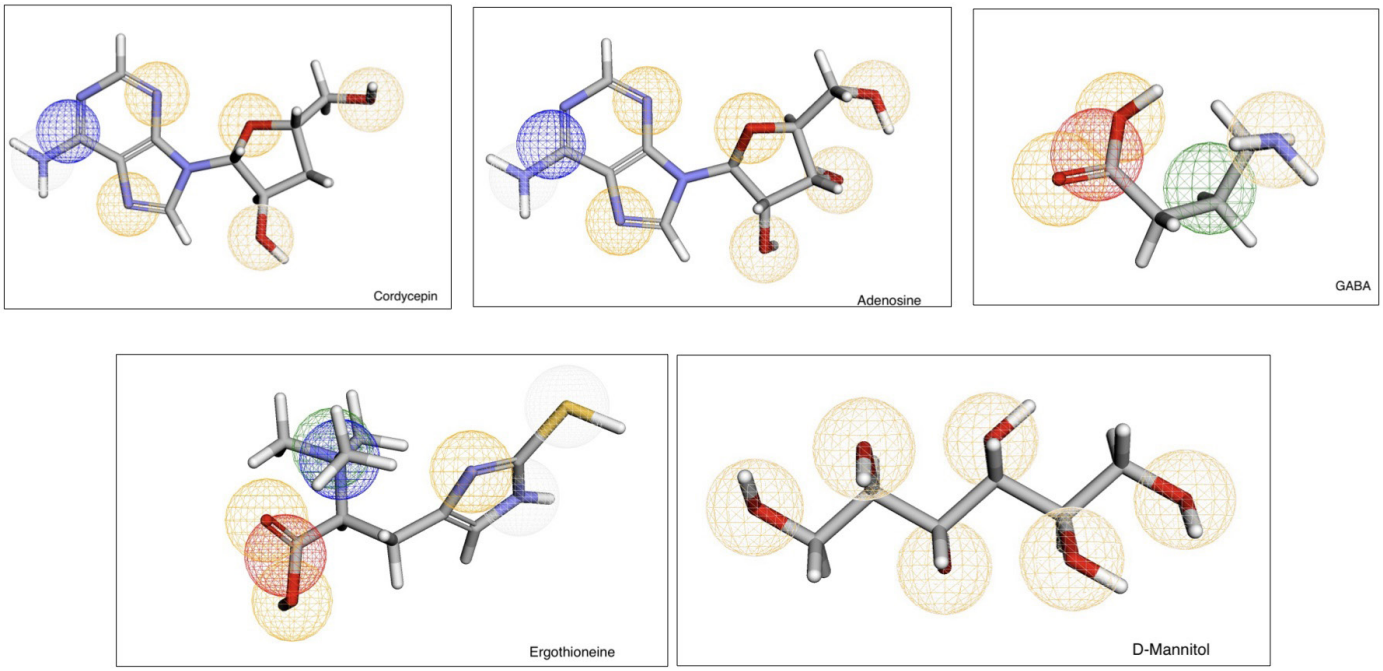


Figure 2: Predicted binding interactions of individual compounds. Representative binding conformations of cordycepin, adenosine, GABA, ergothioneine, and D-mannitol.

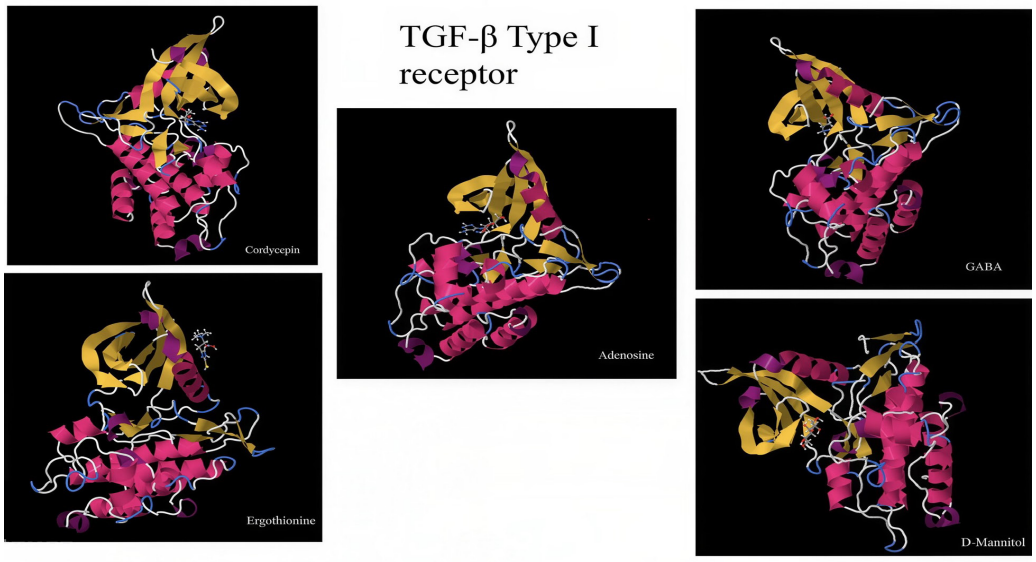


Figure 3: Molecular docking of bioactive compounds from *Cordyceps militaris* with *TGF-β* Type I Receptor (TβRI).

Drug-Likeness and ADME Analysis

The drug-likeness and ADME profiles of selected bioactives were assessed utilizing SwissADME and associated with their binding affinities. Cordycepin and Adenosine preserved favorable pharmacokinetic properties, with bioavailability scores at 0.55 and acceptable logP values, indicating that their structural conformations after docking still adhered to the drug-likeness rules. Compounds like GABA and D-Mannitol presented with minimal change in pharmacokinetic parameters and continued to show weaker properties. Ergothioneine manifested moderate pharmacokinetic characteristics. These findings are detailed in Table 4.

DISCUSSION

Oral Submucous Fibrosis (OSMF) is pathognomonic for advancing and irreversible fibrotic changes of the oral mucosa, largely mediated through TGF-β signaling pathways, which drive fibroblast activation and excessive collagen deposition (Shih *et al.*, 2019). As a result, targeting this pathway might provide a more efficacious treatment strategy by directly interfering with the underlying fibrotic pathogenesis (Shi *et al.*, 2022). The rationale of the present study was to explore natural bioactive compounds capable of inhibiting TGF-β signaling, given its central role in the etiopathogenesis of fibrosis. *Cordyceps militaris*, known for its antifibrotic potential, has been reported to inhibit the TGF-β pathway. The aim of the present *in silico* study was to evaluate the molecular interactions of five major bioactive constituents of *Cordyceps militaris* (Cordycepin, Adenosine, GABA, Ergothioneine, and D-mannitol) with TGF-β Type I (TβRI) and Type II (TβRII) receptors, to assess their potential as natural TGF-β inhibitors and their prospective application in the treatment of OSMF. Since, OSMF exhibited a relatively high malignant transformation risk among oral potentially malignant disorders, effective therapeutic intervention is essential, and targeting key pathways such as TGF-β may aid in reducing fibrosis and disease progression (Kumar *et al.*, 2024).

Drug-likeness screening implementing SwissADME revealed which most components complied with Lipinski's rule of five and other major medicinal chemistry filters. Compliance with Lipinski's rule of five, favorable molecular weight, along with hydrogen bonding capacity suggested that these compounds were likely to have adequate oral absorption and bioavailability, which was critical for therapeutic efficacy in targeting fibrogenic pathways in OSMF (Chen *et al.*, 2023). Cordycepin and Adenosine exhibited optimal molecular weight, hydrogen bonding capacity, and bioavailability scores, consistent with previous *in silico* studies evaluating nucleoside-based compounds as antifibrotic or cytokine-modulating agents (Songprakhon *et al.*, 2024). This was further supported by experimental evidence, where Cordycepin has been shown to consistently inhibit cell migration and cellular inflammation across multiple studies (Radhi *et al.*, 2021), highlighting its functional relevance despite being a small nucleoside molecule with favorable pharmacokinetic properties (Radhi *et al.*, 2021). A comparable ADMET based study by Saritha *et al*; evaluated multiple phytoconstituents for drug-likeness using SwissADME and pkCSM, demonstrating that early computational profiling effectively shortlisted bioactive natural compounds with favorable pharmacokinetic potential despite minor Lipinski violations (Saritha *et al.*, 2024). Similar findings were reported with acceptable pharmacokinetic properties for herbal compounds such as curcumin despite minor rule violations, the present study also found that most *Cordyceps militaris* bioactive compounds showed favorable drug-likeness and ADME profiles with minimal violations (Rai *et al.*, 2022; Rahayu *et al.*, 2024). Collectively, these ADME and drug-likeness evaluations supported the selection of *Cordyceps militaris*

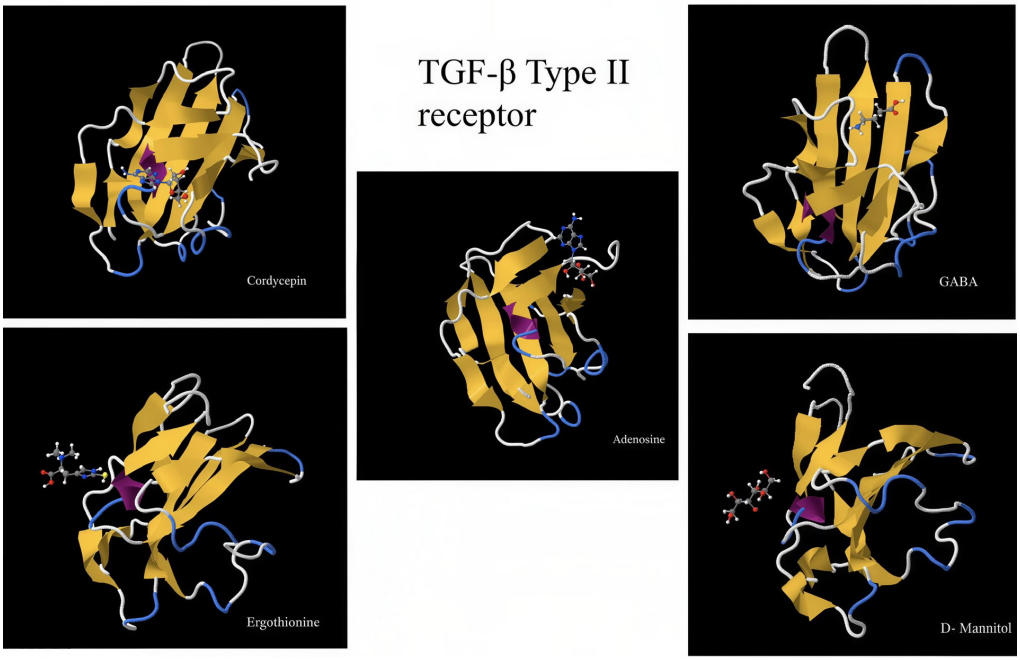


Figure 4: Molecular docking of bioactive compounds from *Cordyceps militaris* with TGF-β Type II Receptor (TβRII).

Table 2: SwissADME analysis of *Cordyceps militaris* bioactive compounds. Molecular weight, binding energy (kcal/mol), bioavailability score, and Topological Polar Surface Area (TPSA) of each compound with TGF-β type II Receptor (TβRII).

TGF-β type II Receptor (TβRII)				
Name	**Molecular weight**	**Binding energy**	**Bioavailability**	**TPSA**
Cordycepin	251.24	-6.4	Low	112.33
Adenosine	267.24	-6.9	Low	142.56
GABA	103.12	-4	High	63.32
Ergothionine	229.3	-6.3	Moderate	104.74
D-Mannitol	182.17	-4.2	High	121.38

bioactives as lead compounds for *in silico* docking studies aimed at TGF-β inhibition in OSMF.

High-resolution crystal structures of TGF-β1 Receptor (TβRI) and TGF-β2 Receptor (TβRII) were extracted from Protein Data Bank and developed using docking analysis by standard protein preprocessing steps (Leonardo-Sousa *et al.*, 2025). Identification of binding pockets was guided by previous structural and functional studies describing key regulatory regions involved in ligand recognition and signal transduction (Rai *et al.*, 2022). Targeting these pockets was specifically pertinent, as inhibition at the receptor level can effectively disrupt downstream fibrogenic signaling cascades. Comparable *in silico* studies targeting TGF-β type I and type II receptors in Oral Submucous Fibrosis have employed similar docking workflows, ligand screening strategies, and active site identification approaches, further supporting the validity of the selected binding regions in the present study (Rahayu *et al.*, 2024).

In silico docking assessment demonstrated that Cordycepin and Adenosine reported the highest strength of binding affinities toward both TGF-β Type I Receptor (TβRI) and TGF-β Type II Receptor (TβRII) among tested compounds. Cordycepin exhibited binding energies of -6.7 kcal/mol and -6.4 kcal/mol, while Adenosine displayed slightly higher affinities of -7.1 kcal/mol and -6.9 kcal/mol against TGF-β Type I Receptor (TβRI) and TGF-β Type II Receptor (TβRII), respectively. These findings were consistent with previous studies reporting the antifibrotic potential of *Cordyceps militaris*-derived compounds through modulation of *TGF-β* signaling pathways. Notably, the binding affinities observed in the present study were comparatively higher, suggesting stronger receptor interactions (Yu *et al.*, 2025). Showed that natural compounds targeting the TGF-β signaling pathway possessed significant structural diversity and multitarget inhibitory potential, while Li *et al*, emphasized on the ability of herbal bioactive molecules to modulate TGF-β-driven pathological pathways with favorable safety profiles (Li *et al.*, 2022). In agreement with these observations, the present study demonstrated that nucleoside derivatives of *Cordyceps militaris* exhibited strong and stable interactions with TGF-β receptors, with binding energies predominantly within the

Table 3: Physicochemical properties of the selected compounds, including hydrogen bond donors, hydrogen bond acceptors, hydrophobic groups, and charge characteristics.

Alkaloid	Hydrogen acceptor	Hydrogen donor	Hydrophobic group	Positive ion	Negative ion
Cordycepin	5	3	0	1	0
Adenosine	6	4	0	1	0
GABA	3	1	1	0	1
Ergothionine	3	2	1	1	1
D-mannitol	6	6	0	0	0

Table 4: Drug-likeness and pharmacokinetic properties predicted by SwissADME. Lipinski, Ghose, Veber, Egan, and Muegge rule assessments along with predicted bioavailability scores for each compound.

Alkaloid	Lipinski	Ghose	Veber	Egan	Muegge	Bioavailability score
Cordycepin	Yes; 0 violation	No; 1 violation: WLOGP<-0.4	Yes	Yes	Yes	0.55
Adenosine	Yes; 0 violation	No; 1 violation: WLOGP<-0.4	Yes	No; 1 violation; TPSA>131.6	Yes	0.55
GABA	Yes; 0 violation	No; 3 violations: MW<160 MR<40 #atoms<20	Yes	Yes	No;3 violations: XLOGP3<-2 MW<200 #C<5	0.55
Ergothioneine	Yes; 0 violation	Yes	Yes	Yes	Yes	0.55
D-Mannitol	Yes; 1 violation: NHorOH>5	"No;2 violations: WLOGP<-0.4 MR<40"	Yes	Yes	No;3 violations: XLOGP3<-2 MW<200 H-don>5	0.55

range of -6 to -7.1 kcal/mol, which fell within the established range (-6 to -8 kcal/mol) indicative of favorable ligand-receptor binding affinity (Ullah *et al.*, 2024). Ergothioneine demonstrated moderate binding affinities (~-6.3 kcal/mol), whereas GABA and D-mannitol exhibited weaker interactions. A similar selective pattern was reported in large-scale *in silico* phytochemical screening for type 2 diabetes mellitus (Subhani *et al.*, 2020), where only a few plant-derived compounds showed strong binding to DPP4 despite most candidates exhibiting moderate to weak interactions consistent with the present findings in which only specific *Cordyceps militaris* bioactives demonstrated stronger interactions with TGF-β receptors.

Visualization of the docked complexes revealed that Cordycepin and Adenosine formed multiple hydrogen bonds and stabilizing interactions within the binding pockets of TGF-β receptors. These interactions involved key polar and charged residues, contributing to stable protein-ligand complexes and explaining their favorable binding energies. Hydrogen bonding and ionic interactions were well-established factors promoting ligand-receptor stability and played a crucial role in enhancing binding affinity in molecular docking studies (Millan-Casarrubias., 2025) Ergothioneine also exhibited strong hydrogen bonding patterns, while GABA and D-mannitol showed limited contact within the binding site. Similar hydrogen bond-mediated interactions and electrostatic stabilization involving ergothioneine have been reported in *in silico* docking studies, where the compound localized near catalytic or NAD^+-binding regions and exhibited moderate binding energies (-4.7 to -2.5 kcal/mol), indicating functionally relevant transient interactions (Parvathi *et al.*, 2025). Such interaction patterns provided mechanistic insight into the inhibitory potential of *C. militaris* bioactive compounds.

Post-docking ADME reassessment indicated that Cordycepin and Adenosine retained favorable pharmacokinetic properties, with stable bioavailability scores and acceptable lipophilicity, suggesting that their docked conformations remained compatible with drug-likeness requirements (Tsai *et al.*, 2010) for treatment of Oral submucous fibrosis (Natarajan *et al.*, 2024; Yadalam *et al.*, 2025). Ergothioneine maintained a moderate yet balanced ADME profile, while GABA and D-mannitol showed minimal changes but continued to exhibit comparatively weaker drug-like characteristics (Arumuganainar *et al.*, 2024). Most compounds showed no violations of Lipinski's rule of five, reinforcing their potential for oral administration. Similar post-docking validation approaches have been reported in *in silico* antifibrotic studies,

where compounds demonstrating strong binding affinity also retained favorable pharmacokinetic profiles (Rahayu *et al.*, 2024; Shree *et al.*, 2026).

Overall, the present findings were consistent with the reported antifibrotic and anti-inflammatory potential of *Cordyceps militaris* by demonstrating inhibitory potential against key fibrogenic cytokines. Among the evaluated compounds, Cordycepin and Adenosine emerged as the most promising candidates, combining strong binding affinity, favorable molecular interactions, and acceptable pharmacokinetic properties. Due to the malignant transformation potential of OSMF, targeting key pathways such as TGF-β may offer added benefits by not only attenuating fibrosis but also limiting disease progression, supporting the translational relevance of these findings. This integrative *in silico* evaluation highlighted the feasibility of developing *C. militaris*-derived bioactive compounds as targeted antifibrotic therapeutics for Oral Submucous Fibrosis. Considering the malignant transformation potential of OSMF, the observed inhibition of TGF-β signaling by the evaluated compounds enhanced their therapeutic relevance, indicating a potential role in modulating both fibrosis and disease progression.

The present *in silico* study provided useful predictions but did not reflect the entire complexity of biological and metabolic stability of the compounds *in vivo*. The static protein structures used may not represent the dynamic conformational changes occurring under physiological conditions. In addition, without *in vitro* or *in vivo* experiments, the validation of the findings and clinical relevance was limited.

CONCLUSION

The present *in silico* study suggested that bioactive compounds from *Cordyceps militaris*, particularly Cordycepin and Adenosine, demonstrated high binding affinities toward TGF-β Type I Receptor (TβRI) and TGF-β type II receptor (TβRII), indicating prospective anti-fibrotic effects. Ergothioneine also displayed favorable outcomes, while GABA and D-Mannitol showed moderate potential. These results reinforced the traditional use of *C. militaris* and highlighted its remedial capability in managing Oral Submucous Fibrosis, warranting further experimental validation.

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ABBREVIATIONS

OSMF: Oral submucous fibrosis; **TGF-β:** Transforming Growth Factor-beta; **TβRI:** Transforming Growth Factor-beta Type I Receptor; **TβRII:** Transforming Growth Factor-beta Type II Receptor; **ADME:** Absorption, Distribution, Metabolism, and Excretion; **GABA:** Gamma-aminobutyric acid; **HPV:** Human papilloma virus; **ECM:** Extracellular matrix; **PDB:** Protein Data Bank; **TPSA:** Topological polar surface area; **logP:** Lipophilicity; **SMILES:** Simplified Molecular Input Line Entry System; **MW:** Molecular weight; **MR:** Molar Refractivity; **SDF:** Structure Data File.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

SUMMARY

This study investigated the potential of bioactive compounds from *Cordyceps militaris* as inhibitors of the TGF-β signaling pathway, a key driver of Oral Submucous Fibrosis (OSMF). Through *in silico* molecular docking and ADME analysis, the study identified Cordycepin and Adenosine as promising therapeutic candidates. These compounds demonstrated high binding affinities for TβRI and TβRII receptors while maintaining favorable pharmacokinetic profiles and drug-likeness. The findings suggest that *C. militaris* derivatives could serve as targeted antifibrotic agents, warranting further experimental validation for the management of OSMF.

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