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# Computational Prediction of *Cinnamomum zeylanicum* Bioactive Compounds as Potential Antifungal by Inhibit Biofilm Formation of *Candida albicans*

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**Abstract**

The issue of *Candida albicans* resistance to antifungal agents in the management of candidiasis has emerged as a significant concern, necessitating the development of novel antifungal alternatives to ensure efficacy in the treatment of candidiasis. A notable manifestation of *C. albicans* pathogenicity is the development of biofilms, which are facilitated by the presence of certain genes known as agglutinin-like sequence (ALS) genes, specifically ALS1 and ALS3. The utilisation of *Cinnamomum zeylanicum* extract as a potential alternative antifungal agent has promise in addressing fungal resistance, particularly in the context of *C. albicans*, for the treatment of candidiasis. Bioactive compounds such as cinnamonaldehyde, cyclopentane, eugenic acid, hexadecenoid acid and Pyrantel Hydrochloride are present in *C. zeylanicum.* This research investigates the mechanistic plausibility of the bioactive chemicals found in *C. zeylanicum* as agents for combating candidiasis. The study specifically examines the inhibitory effects of these compounds on the Agglutinin-like sequence-1 (ALS1) and ALS3 proteins. The study employs a combination of molecular docking and molecular dynamics simulations to elucidate the complex interactions between the bioactive constituents of *C. zeylanicum* and ALS1/ALS3 proteins. The ligand exhibited notable binding affinity towards the molecules Cinnamaldehyde, Pyrantel Hydrochloride and Hexadecenoic Acid derived from *C. zeylanicum*, as seen by their binding affinity values ranging from –4.873 to –6.03 kcal/mol. This indicates their potential effectiveness in altering the activities of the target proteins. The investigation reveals distinct interaction patterns between each chemical and the proteases. Significantly, Pyrantel Hydrochloride establishes binding interactions with essential catalytic residues in both ALS1 and ALS3. The stability of the Pyrantel Hydrochloride-ALS1/ALS3 complexes is further supported by molecular dynamics simulations, which suggest that these complexes possess a strong inhibitory capacity. The preservation of protein structures appears to be minimally impacted, indicating a prolonged inhibitory effect. The effectiveness of Pyrantel Hydrochloride is emphasized by the numerous hydrogen bonds that are formed and confirmed using free-binding energy calculations. Collectively, the results highlight the plausibility of *C. zeylanicum* anti-candidiasis action, principally mediated through the inhibition of ALS1 and ALS3. This activity is predominantly facilitated by Pyrantel Hydrochloride. This

comprehensive understanding provides a valuable foundation for the development of innovative antifungal strategies in the battle against *C. albicans* infections.

**Keywords:** Agglutinin-like sequence, Antifungal, Biofilm formation, Molecular docking, Molecular dynamics

## Introduction

Oral candidiasis is an opportunistic infection that most commonly occurs in people living with immunocompromised conditions such as HIV [1,2]. *C. albicans* has been identified as the species most responsible for 90 % of invasive fungal infections worldwide [3], including oral candidiasis, which occurs when the immune system is compromised [2,4]. *C. albicans* is also recognized as the 4$^{th}$ main cause of infection in immunocompromised hospital patients [5]. If the host's immune system or normal microbiota is perturbed, *C. albicans* can cause a variety of diseases [6,7]. Biofilm formation is associated with the majority of *C. albicans*-caused infections [8]. The formation of surface-adhering biofilms is essential for *C. albicans* virulence [9]. *C. albicans* has greater resistance to antimicrobials when it exists as a biofilm, compared to its planktonic cell state [10]. Consequently, biofilm formation significantly increases therapy failure, morbidity and mortality [9]. The escalation of antimicrobial resistance in *C. albicans* is significantly impacted by virulence factors.

The capacity of *C. albicans* to successfully invade various host environments is facilitated by a broad spectrum of virulence factors and fitness characteristics. Several properties are regarded as virulence factors, including as the morphological transition from yeast to hyphal forms, the presence of adhesins and invasins on the cell surface, thigmotropism, biofilm development, phenotypic switching and the production of hydrolytic enzymes [11,12]. *C. albicans* has a unique set of proteins called adhesins that let it stick to other *C. albicans* cells, as well as to different bacteria, non-living surfaces and host cells [11,13,14]. The adhesins of *C. albicans* possesses 2 distinct sets of protein families that are involved in adhesion activity in its filamentous form [15]. These protein families are known as ALS (ALS1-7 and ALS9) [11,15]. Among the proteins associated with ALS, ALS3 plays a pivotal function in the process of adhesion. The proteins mentioned above are the resulting products of the ALS1 and ALS3 genes, as documented in references [11,16,17]. To date, the ALS1 and ALS3 genes have been found to play a significant role in the pathogenicity of *C. albicans*, namely in terms of facilitating adhesion and promoting biofilm formation. Consequently, these genes have emerged as primary targets for antifungal therapy strategies aimed at combating candidiasis.

Currently, only 5 compounds are used to treat Candida infections: Polyenes, allylamines, azoles, fluoropyrimidine and echinocandine [18]. Resistance to antifungal medications such as fluconazole, voriconazole and amphotericin is increasing [19,20]. Over the past 5 years, *C. albicans* resistance to azole, echinocandin and amphotericin B in mucosal-infected patients has developed gradually. Increasing antifungal drug resistance and the inherent toxicity of these drugs have diminished the efficacy of treatment [21]. In spite of heightened consciousness and improved therapeutic approaches, the emergence of drug resistance in fungal diseases persists, presenting a significant peril to worldwide public health and healthcare infrastructures [22]. Therefore, there is an urgent need to discover novel antifungal agents with fewer side effects and increased antifungal activity to treat fungal infections.

The demand for novel antifungal agents has prompted scientists to explore the potential of natural compounds, such as *C. zeylanicum*, which are recognized for their phytochemical composition that exhibits inhibitory effects against fungal development. Notably, (E)-cinnamaldehyde and eugenol have been identified as key bioactive constituents in *C. zeylanicum* with antifungal properties [23,24]. Furthermore, the preference for traditional medicinal plant therapy arises from its ability to generate fewer unwanted effects and exhibit greater efficacy [25]. The authors [26] argue that the incorporation of phytochemicals, either as standalone treatments or in combination with conventional drugs, could offer a more beneficial therapeutic strategy for fungal infections. This is due to the reduced toxicity and minimal ecological impact associated with the use of phytochemicals.

Regarding this matter, it is worth noting that alcoholic extracts derived from medicinal plants have demonstrated potential for enhanced extraction efficacy. Notably, certain extracts have exhibited superior antifungal properties compared to conventional antibiotics, the documentation has been provided regarding the comprehensive observation and reporting of the antifungal activity exhibited by aqueous extracts [27]. For example, phytochemical compounds in the form of flavonoids have been documented to show antifungal properties against many fungal diseases by disrupting plasma membrane integrity, inhibiting DNA, RNA and protein synthesis and damaging mitocondria [28].

Currently, in the field of research, computational approaches known as *in silico* techniques are employed to make predictions regarding the efficacy of phytobiotics in combating antimicrobial activity [23,29]. The study conducted by [30], utilized *in silico* methods to identify possible molecular targets for curcumin, including superoxide dismutase, catalase and isocitrate lyase. A computer analysis conducted by [23] has identified hexadecenoic acid as a possible inhibitor of Secreted Aspartyl Proteinase (SAP) 5 and SAP 6 which are responsible for the formation of *C. albicans* hypae. However, the essential oil found in cinnamon's has the ability to operate as an antifungal agent and can hinder the virulence factors of *C. albicans*, such as ALS and SAP, both *in vitro* and *in vivo* techniques [31,32]. The aim of this study is to predict the effectiveness of the phytochemical compounds present in *C. zeylanicum*, which exhibit potential antifungal activities, specifically in their capacity to impede the formation of biofilms controlled by ALS1 and ALS3.

## Materials and methods

### Ligand structure retrieval

The bioactive compounds of cinnamon were obtained from PubChem, as reported by [33] and referenced from the earlier study conducted by [23]. The source for the bioactive compounds is available at: https://pubchem.ncbi.nlm.nih.gov/. The 5 bioactive chemicals under consideration are Cyclopentane (CID: 7296), Cinnamaldehyde (CID: 637511), Eugenol (CID: 3314), Hexadecenoid Acid (CID: 13105359) and Pyrantel Hydrochloride (CID: 6365307). The compound (Z)-N-(5-acetyl-4-phenyl-1, the compound with the CID number 97635812, specifically -3-(1,3-benzodioxol-5-yl) prop-2-enamide, was employed as a control in the study.

### Protein sequence retrieval and structural modeling

The protein sequences of ALS1 and ALS3 were obtained from UniProt, with the accession numbers Q5A8T4 and Q59L12, respectively. The protein ALS1 and ALS3 were subjected to modelling using the SwissModel platform (swissmodel.expasy.org/) as described by [34]. The x-ray diffraction structure of ALS3 (PDB ID: 4LEE) was utilized as the reference template for the modelling process. The 3-dimensional (3D) structure of the models was acquired and subsequently saved in .pdb format.

### Molecular docking

The molecular docking methodology was executed following the established approach as stated in a previous study by [35]. The compounds underwent energy minimization using the Open Babel software [36] within the PyRx 0.9.5 interface [37], employing the universal forcefield, before being designated as the ligands [38]. Subsequently, the macromolecular nature of proteins was established by determining their 3D structure. The grid configuration for precise docking was established based on prior research conducted by [39], (**Table 1**). The binding pose prediction and estimation of binding energy were performed using Vina 1.2.3 [37,38] within the PyRx interface, with the exhaustiveness set to its default value. The ligand conformation that was acquired was subsequently subjected to complexation and analysis using Biovia Discovery Studio. The complexes with the lowest binding energy were subsequently chosen for molecular dynamics modelling.

**Table 1** The grid box position for specific molecular docking.

| Proteins | Center | | | Dimensions (Å) | | |
|---|---|---|---|---|---|---|
| | X | Y | Z | X | Y | Z |
| **ALS1** | 20.051 | 2.205 | 66.492 | 25.774 | 34.758 | 28.316 |
| **ALS3** | 18.546 | 8.483 | 67.383 | 36.740 | 28.600 | 30.568 |

### Molecular dynamics

Simulation of molecular dynamics was performed using YASARA Structure version 23.4.25.W.64 [42] for 20 20.00 nanoseconds with 401 snapshots and the AMBER14 force fields [43]. Simulations were performed under physiological conditions as follows [44]: 310 °K of temperature; pH 7.4; 0.9 NaCl concentration; 0.997 g/mL of water density; 1 bar pressure. Simulation was performed under Cubic cell shape. The simulation trajectories then analyzed using YASARA macros, i.e., md_analyze.mcr and md_analyzeres.mcr. Structural dynamics were described as Root-Mean-Square Deviation (RMSD) of atomic positions and Root-Mean-Square Fluctuation (RMSF) of per residue position in Angstrom (Å) unit.

## Results and discussion

### Interaction of cinnamon's bioactives against ALS1 and ALS3

Molecular docking was conducted to investigate the interactions of various compounds with ALS1 and ALS3. Among the tested compounds, the compound with the lowest binding affinity was found to be Pyrantel Hydrochloride, with energy values of –5.822 kcal/mol for ALS1 and –6.03 kcal/mol for ALS3. Interestingly, the 2nd lowest binding affinities for ALS1 and ALS3 were exhibited by cinnamaldehyde and Eugenol, respectively, with energy values of –5.484 and –5.616 kcal/mol. Comparable binding affinities to the control compound were shown by these bioactive compounds (**Table 2**).

**Table 2** Binding affinity of docked bioactive compounds (left) with proteins (top).

| Ligand | Binding affinity (kcal/mol) | |
|---|---|---|
| | **ALS1** | **ALS3** |
| **Control** | –8.582 | –8.502 |
| **Cinnamaldehyde** | –5.484 | –5.437 |
| **Cyclopentane** | –4.04 | –3.993 |
| **Eugenol** | –5.476 | –5.616 |
| **Hexadecenoid acid** | –4.873 | –5.049 |
| **Pyrantel Hydrochloride** | –5.822 | –6.03 |

Pyrantel Hydrochloride has the highest interaction (15 interactions) with ALS1, identical to the drug control. The 2nd highest interaction was cinnamaldehyde with 12 interactions (**Figure 1**). The same results were also applied to ALS3, Pyrantel Hydrochloride has highest interaction with ALS3 with a comparable number (14 interaction) with the drug control (16 interaction) and the 2nd highest interaction was occupied by Eugenol with 12 interactions (**Figure 2**). The process of docking a molecule within a protein binding site is a reliable method for determining the accurate binding orientation from a set of multiple expected orientations of a substance [45].

Further analysis revealed that specific amino acid residues were involved in the interactions between ALS1 and ALS3 and Pyrantel Hydrochloride. For ALS1, the interacting residues were identified as PRO46, VAL285, THR313 and TYR315. However, for ALS3, a higher number of interacting residues were identified, including TYR38, PRO46, LYS76, ALA133, ARG188, THR313 and TYR315. Interestingly, it was observed that THR313 was the only common amino acid residue that interacted with Pyrantel Hydrochloride and the control compound in ALS1. Similarly, ALS1 also interacted with Cinnamaldehyde, with the only difference being the residue SER187 (**Figure 1**). For ALS3, interactions with Pyrantel Hydrochloride were found with 3 specific amino acid residues: TYR38, PRO46 and ALA133. In contrast, only 1 identical interacting residue with the control compound was shared by Eugenol (**Figure 2**). In these interactions, 3 main types of interactions play significant roles: Hydrogen bonds, hydrophobic bonds and van der Waals interactions. These interactions help stabilize the binding of compounds to the receptors ALS1 and ALS3. Hydrogen bonds are the most influential in protein-ligand binding [43-44], but the presence of other interactions like hydrophobic bond and Van der Waals interactions further stabilizes the binding [43,45], resulting in better inhibitory activity against ALS1 and ALS3.

Previous studies have demonstrated the significance of ALS proteins in *C. albicans*' adhesion to host cell receptors through their N-terminal adhesive domains, and by forming amyloid-like structures, facilitating tight adherence to epithelial and mucosal surfaces. These proteins are also involved in biofilm formation, making them potential targets for antifungal therapy [46-49]. Some research has explored compounds like N-(oxazolylmethyl)-thiazolidindione which demonstrated binding to residues THR78, ALA186, SER187 and ARG188 of ALS1 protein, as well as SER187 and TYR288 of ALS3 [39]. Additionally, zinc oxide nanoparticles (ZnO-np) have shown promise in preventing fungal adhesion to surfaces [15,50]. Nevertheless, the discovery of Pyrantel Hydrochloride as a potential ALS inhibitor represents a novel finding with potential implications for future drug development and treatment strategies.

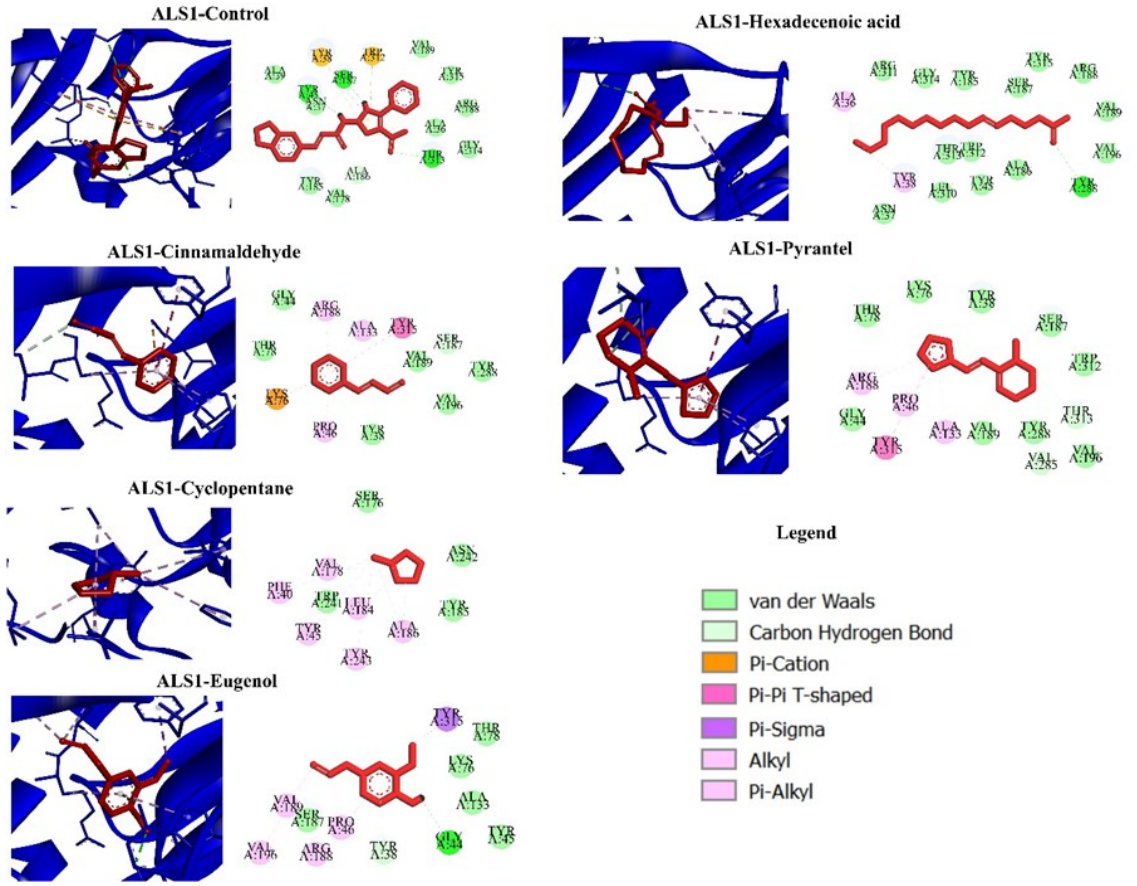


**Figure 1** Structural visualization of the ALS1-Cinnamon Compounds according to the docking result. ALS1 was visualized in blue ribbon, while the Cinnamon Compounds were displayed as red sticks. Amino acids interactions were visualized by colored balls with different color representing each chemistry interaction with the ligand's structure.

**Structural stability of selected complexes**

*In silico* screening shortlisted Pyrantel Hydrochloride (Pyn-HCl) and Eugenol for ALS3, and Pyn-HCl with Cinnamaldehyde for ALS1, as potential inhibitors. However, docking's inherent static nature necessitates molecular dynamics (MD) simulations to elucidate the binding stability and structural robustness of ALS1/ALS3-inhibitor complexes in a dynamic environment. Protein backbone Root Mean Square Deviation (RMSD) and individual residue Root Mean Square Fluctuation (RMSF) will be employed to evaluate complex stability. Lower RMSD and RMSF values correspond to a more rigid and stable protein-ligand interaction [33,42,50]. Consequently, these 4 complexes will undergo MD simulations for a deeper understanding of binding stability compared to control molecules.

The stability of the receptor plays a crucial role in determining the enthalpic and entropic contributions to the overall stability of the protein-ligand complex [51]. To assess this stability, key parameters such as the RMSD of backbone atoms and the RMSF are commonly employed. The RMSD measures the overall stability of the protein structure by examining the positions of backbone atoms, while the RMSF provides insights into the stability at the per-residue level [54]. According to simulation results, the binding of Pyrantel Hydrochloride and Cinnamaldehyde did not significantly influence the structural stability of ALS1. This conclusion is supported by the RMSD and RMSF data, which revealed no major differences in the stability of the ALS1 backbone atoms (**Figure 3(A)**) or per-residue fluctuations (**Figure 3(B)**) between the ALS1-Pyrantel Hydrochloride and ALS1-Cinnamaldehyde complexes. Although there were some fluctuations observed at residues CYS 256 and THR 257 in the ALS1-Pyrantel Hydrochloride complex, these residues do not participate in ligand binding. Therefore, the binding of Pyrantel Hydrochloride and Cinnamaldehyde to ALS1 occurs in a stable conformation, enhancing the overall binding stability and inhibitory activity [52].

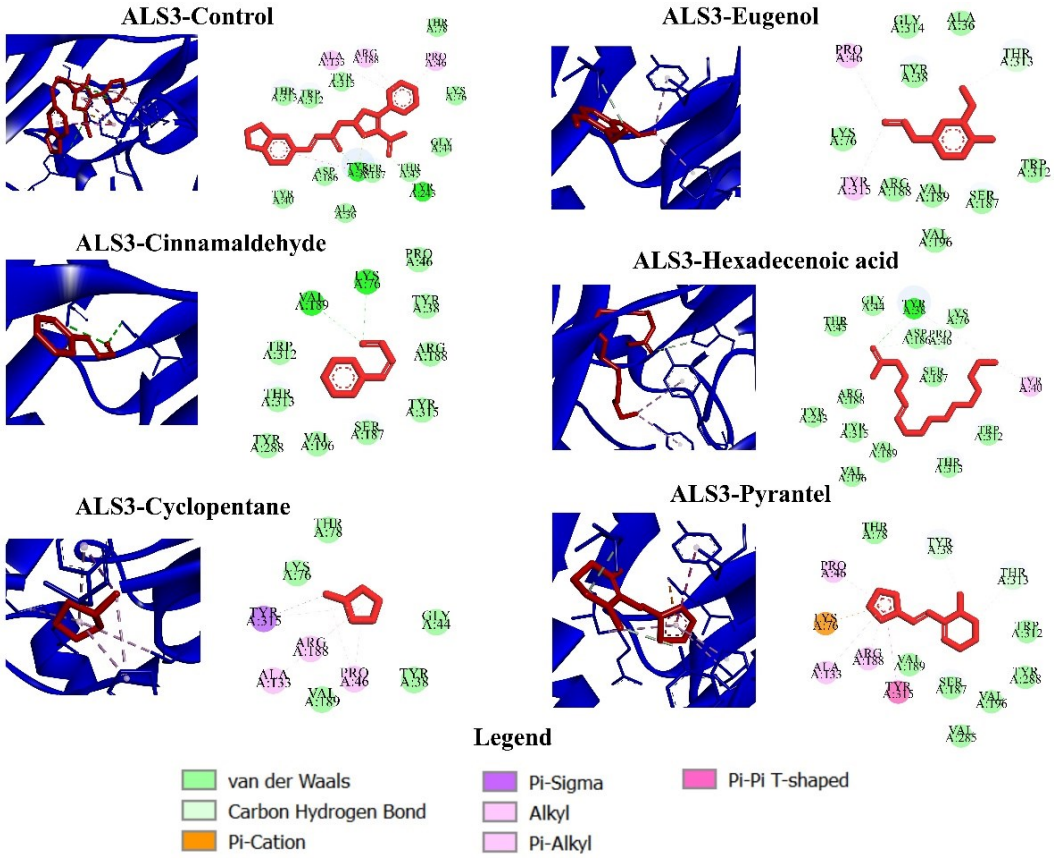


**Figure 2** Structural visualization of the ALS3-Cinnamon Compounds according to the docking result. ALS3 was visualized in blue ribbon, while the Cinnamon Compounds were displayed as red sticks. Amino acids interactions were visualized by colored balls with different color representing each chemistry interaction with the ligand's structure.

To assess the binding stability of the ligand within the protein-ligand complex, the RMSD of ligand movement was analyzed. A low and stable RMSD value indicates that the ligand maintains its position during interaction with the protein, reflecting the overall stability of the complex [33]. Results demonstrated that both Cinnamaldehyde and Pyrantel Hydrochloride exhibited more stable conformations compared to the control molecule (**Figure 3(C)**), suggesting fewer structural alterations upon binding to ALS1. This stability is further supported by the ligand movement RMSD, where both compounds showed more stable values than the control molecule (**Figure 3(D)**). Although Cinnamaldehyde exhibited slightly more fluctuations, both compounds displayed less movement than the control. These findings suggest that Pyrantel Hydrochloride and Cinnamaldehyde are promising inhibitors of ALS1, owing to their stable binding conformations.

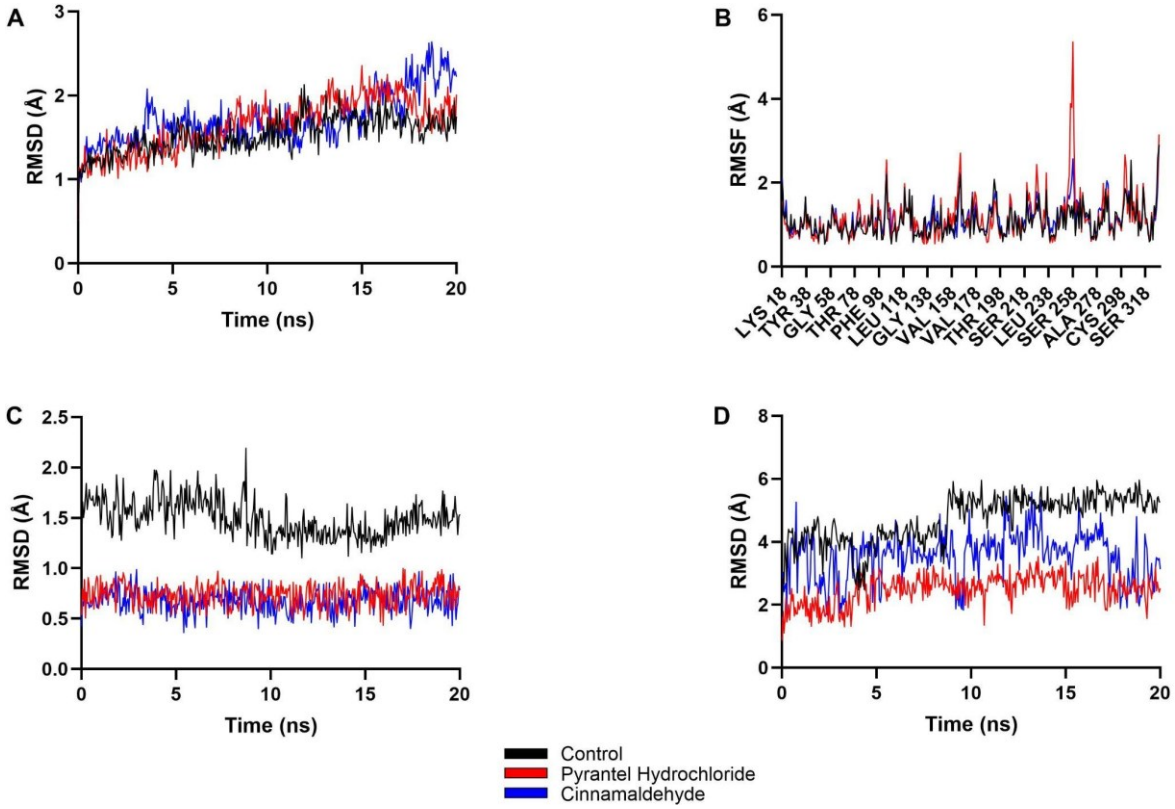


**Figure 3** Structural stability assessment of Pyrantel Hydrochloride and Cinnamaldehyde complexes with ALS1. Protein and complex stability were assessed through the RMSD of backbone atom (A) and RMSF (B), while ligand conformation and binding stability were predicted through the RMSD of ligand conformation (C) and ligand movement (D).

The stability of receptor-ligand complexes is pivotal for effective inhibition, and this is demonstrated by various stability parameters. Pyrantel Hydrochloride and Eugenol, similar to their interaction with ALS1, displayed minimal impact on ALS3's protein conformation stability. This is evidenced by the low RMSD values of backbone atoms (**Figure 4(A)**) and RMSF values (**Figure 4(B)**), indicating minor fluctuations of less than 1 angstrom. Both ALS3-Pyrantel Hydrochloride and ALS3-Eugenol complexes exhibited less fluctuation than the ALS3-control, particularly at residues TYR 220, ASP 222 and THR 283, suggesting more stable conformations upon binding.

Furthermore, the stability of the ligand itself plays a crucial role in its binding efficacy. Evaluating the RMSD of ligand conformation revealed that Pyrantel Hydrochloride, Cinnamaldehyde and Eugenol outperformed the control molecule in terms of conformational stability. This stability reduces the entropic penalty that can hinder complex formation [53], leading to more stable protein-ligand interactions [51]. The RMSD of ligand movement also supported these findings, showing less movement for Pyrantel Hydrochloride and Eugenol compared to the control (**Figure 4(D)**). Despite an initial spike, Eugenol achieved stability after approximately 3 ns, while Pyrantel Hydrochloride maintained consistent stability throughout. This data underscores the high probability of these compounds, particularly Pyrantel Hydrochloride and Eugenol, to inhibit ALS3 activity in a stable conformation and binding.

Moreover, the binding stability of these ligands with ALS3 is consistent with their performance in ALS1 binding. Pyrantel Hydrochloride and Cinnamaldehyde displayed stable conformations, as indicated by the RMSD of ligand movement, which remained lower and more stable compared to the control molecule. This highlights the potential of Pyrantel Hydrochloride, Cinnamaldehyde and Eugenol as promising inhibitors of both ALS1 and ALS3, offering potential preventative or therapeutic benefits against candidiasis.

ALS genes play a crucial role in promoting adherence and initial biofilm establishment, which significantly contributes to antifungal resistance, including resistance to fluconazole. Specifically, ALS1 and ALS3 are associated with antibiotic resistance, likely due to their involvement in biofilm

development [51,52]. Inhibiting ALS1 and ALS3 has been shown to be beneficial in suppressing biofilm formation [57], making these genes critical targets for preventing biofilm formation during candidiasis development.

Based on available data, Pyrantel Hydrochloride, Cinnamaldehyde and Eugenol demonstrate promising inhibitory effects on ALS1 and ALS3. Those compounds exhibited relatively strong binding affinities to ALS1 and ALS3 and interacted with similar residues compared to the control molecules. Moreover, those compounds also minimally affect receptor stability to facilitate stable binding according to the molecular dynamics results. The resulting stable protein-inhibitor complex enhances inhibitory activity against these proteins [58], critical for cell adhesion. Inhibiting ALS1 and ALS3 reduces cellular heterogeneity by disrupting adhesion functions, thereby preventing biofilm formation due to a deficiency in necessary adhesin molecules for cell-to-cell adhesion [59]. However, further *in vitro* and *in vivo* studies are necessary to confirm the inhibitory activity of these bioactive compounds from cinnamon.

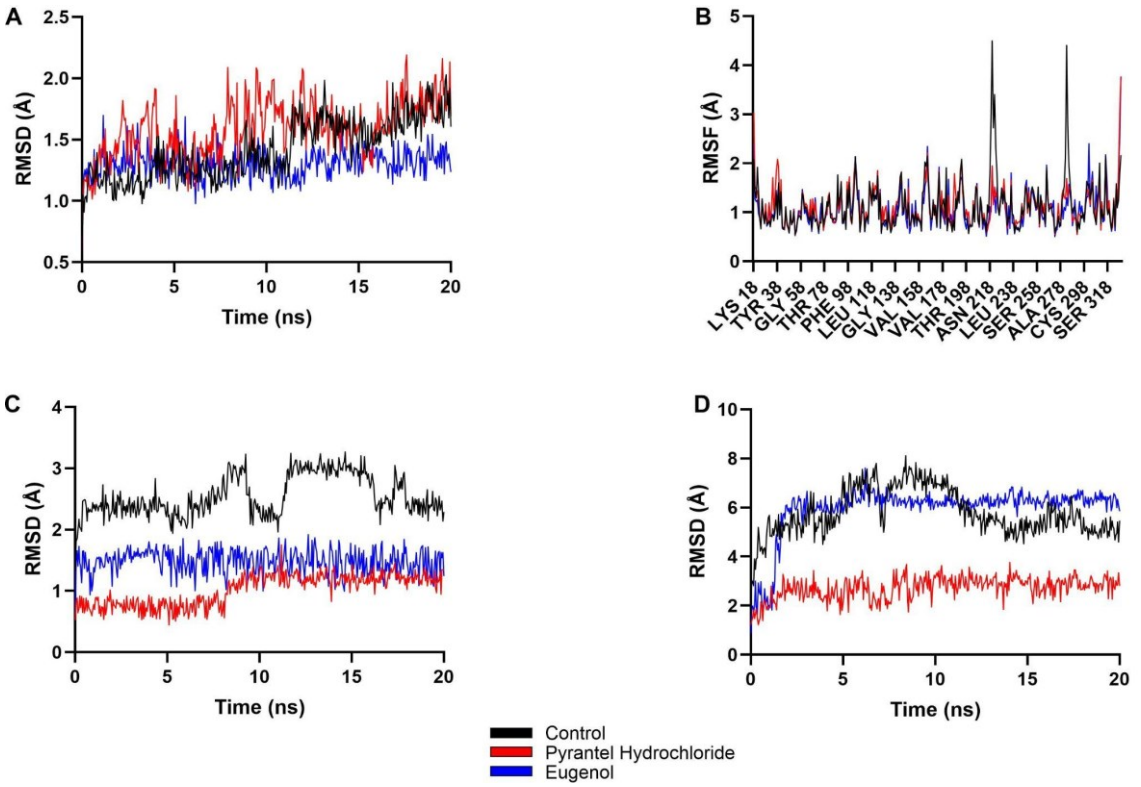


**Figure 4** Structural stability assessment of Pyrantel Hydrochloride and Eugenol complexes with ALS3. Protein and complex stability were assessed through the RMSD of backbone atom (A) and RMSF (B), while ligand conformation and binding stability were predicted through the RMSD of ligand conformation (C) and ligand movement (D).

## Conclusions

The preservation of the protein structures appears to be mostly unaffected, indicating a prolonged inhibitory effect. The effectiveness of Pyrantel Hydrochloride is emphasized by the numerous hydrogen bonds that are formed and confirmed using free-binding energy calculations. The collective findings emphasize the plausibility of *C. zeylanicum*'s anti-candidiasis activity, principally achieved by the inhibition of ALS1 and ALS3. This inhibition is predominantly facilitated by Pyrantel Hydrochloride. This comprehensive understanding provides a valuable foundation for the development of innovative antifungal strategies in the battle against *C. albicans* infections.

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