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Molecular Docking Analysis of Bioactive Compounds from *Cinnamomum zeylanicum* Bark Essential Oil Targeting Chitin Synthase and 1,3-β-Glucan Synthase in Mycotoxigenic Fungi

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Abstract

Mycotoxigenic fungi pose significant threat to food safety and global agriculture, necessitating the development of effective and sustainable natural antifungal agents. Cinnamomum zeylanicum bark essential oil (CBEO) is known for its broad antifungal activity; however, the molecular mechanisms underlying its effects remain poorly understood. In this study, an in silico approach was employed to investigate the inhibitory potential of Gas Chromatography–Mass Spectrometry(GC–MS)-identified CBEO constituents against two essential fungal cell-wall biosynthetic enzymes: chitin synthase (CHS) and 1,3-β-glucan synthase (FKS). Docking analyses were performed using AlphaFold-predicted protein structures and a binding energy threshold of ≤ -6.0 kcal/mol was set as benchmark based on established binding energies of known antifungal inhibitors: nikkomycin Z and caspofungin. Several CBEO compounds exhibited favorable binding affinities, indicating strong interactions with the catalytic regions of both enzymes. Benzyl benzoate (- 6.937 kcal/mol), copaene (-6.692 kcal/mol), and Acetate, cinnamyl ester (-6.311 kcal/mol) showed binding affinities comparable to nikkomycin Z against CHS, while alpha-phellandrene dimer (-8.211 kcal/mol), caryophyllenyl alcohol (-7.686 kcal/mol), and β-caryophyllene (-7.657 kcal/mol) surpassed the reference drug caspofungin against FKS. Interaction analyses revealed that hydrophobic interactions, π-alkyl bonds, and aromatic stacking played dominant roles in stabilizing the ligand-enzyme complexes. These findings indicate that CBEO contains multiple bioactive constituents capable of targeting fungal cell-wall biosynthesis through a multi-target mechanism, supporting its potential application as a natural antifungal agent for improving food safety and mitigating mycotoxin contamination in cereal products.

Keywords: Molecular docking, Chitin synthase, 1,3-beta-glucan synthase, *Cinnamomum zeylanicum,* Mycotoxigenic fungi.

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INTRODUCTION

Mycotoxigenic fungi constitute a major global challenge affecting the safety, nutritional value, and marketability of cereal crops such as maize and soghurm (Eskola *et al.,* 2020). Species belonging to the genera *Aspergillus, Fusarium,* and *Penicillium* are responsible for producing potent mycotoxins including aflatoxins, fumonisins, ochratoxin A, and trichothecenes, which are associated with hepatotoxicity, carcinogenesis, immunosuppression, and growth impairment in humans and animals (Kumar *et al.,* 2022). In addition to health risks, fungal contamination results in substantial economic losses due to reduced crop quality and international trade restrictions (Eskola *et al.,* 2020).

Conventional management depends heavily on synthetic fungicides; however, the increasing emergence of resistant fungal strains, environmental persistence of chemical residues, regulatory constraints, and consumer preference for green alternatives highlight the need for safer antifungal strategies (Fisher *et al.,* 2018). Consequently, natural products, especially plant essential oils, have gained attention as sustainable antifungal agents due to their broad antimicrobial spectrum, biodegradability, and multi-target modes of action (Hyldgaard *et al.,* 2012).

Cinnamomum zeylanicum bark essential oil (CBEO), is well known for its broad antimicrobial spectrum, attributed largely to phenylpropanoids and terpenoids such as cinnamaldehyde, eugenol, benzyl benzoate, and various terpene dimers (Mutlu-Ingok *et al.,* 2020). Numerous studies demonstrate that CBEO constituents can disrupt fungal cell membranes, alter oxidative stress pathways, and impair cell-wall structure (Zhang *et al.,* 2017). However, despite extensive experimental data, the precise molecular targets underlying these antifungal effects require deeper mechanistic exploration.

The fungal cell wall is a dynamic extracellular matrix composed primarily of chitin and β-1,3-glucans. The biosynthesis of these polymers is mediated by two essential enzymes: chitin synthase (CHS) and 1,3-β-glucan synthase (FKS)(Zhang *et al.,* 2017). Mutations or inhibition of these enzymes lead to structural instability, osmotic fragility, and fungal lysis (Lenardon *et al.,* 2010). They are validated antifungal targets, as demonstrated by the clinical success of echinocandins (targeting FKS) and the experimental agent nikkomycin (targeting CHS) (Walker *et al.,* 2015). Identifying natural inhibitors of CHS and FKS may therefore yield promising antifungal candidates.

Computational methods such as molecular docking provide valuable insights by predicting ligand binding affinity, identifying amino acid interactions, and screening natural product libraries before laboratory validation (Morris and Lim-Wilby, 2008; Ferreira *et al.,* 2015). Docking is especially useful for essential oil constituents, which often act synergistically and interact with multiple cellular targets (Ferreira *et al.,* 2015). This study aimed to examine the potential inhibitory interactions between GC–MS-identified CBEO constituents and fungal CHS and FKS thereby providing mechanistic insight into their antifungal potential.

MATERIALS AND METHODS

Ligand Selection and Preparation

Compounds identified from Gas Chromatography–Mass Spectrometry(GC–MS) analysis of *C. zeylanicum* bark essential oil were selected for docking studies. Based on the ligands recognized, the 2D structures were obtained from the PubChem database in Structure Data File (SDF). The geometry optimization of each ligand molecule was performed by using

Avogadro software using Force Field MMF94, algorithm steepest descent, 500 steps. After the PDF format of each file was subjected to AutoDock Tools 1.5.6 and converted to PDBQT format.

Protein Preparation

The structures of the two proteins used chitin synthase (Protein ID: A0A370CDA0) and 1,3-beta-glucan synthase component of FKS1 (Protein ID: AF-A2QLK4-F1-v4) were retrieved from the AlphaFold server (https://alphafoldserver.com/). Structural integrity was evaluated using the Ramachandran plot.

Molecular Docking

The European instance of the Galaxy server version 25.0.2.dev0 (www.usegalaxy.eu) was used for the molecular docking studies following method described by Bray (2023). Grid box parameters were defined to encompass the catalytic regions of each enzyme. Docking simulations generated binding affinity scores (kcal/mol), and the best-ranked poses were selected for further analysis.

Nikkomycin Z (a known chitin synthase inhibitor) and caspofungin (an echinocandin that inhibits β-1,3-glucan synthase) were considered as reference drugs for benchmarking. Based on the published binding energy ranges reported by Farhadi *et al.* (2020) and Ran *et al.* (2024), a binding energy threshold of -6.0 kcal mol^{-1} was adopted in this study to identify compounds from *Cinnamomum zeylanicum* essential oil with potentially strong affinity for chitin synthase and 1,3-beta-glucan synthase. Ligands scoring at or below this threshold were considered to have favourable binding interactions relative to the standards.

Analysis of Docking Results

The visualization of the molecular interactions and binding conformation of each protein and ligands was done using discovery studio visualizer v21.1.0.20298 (Dassault Systemes Biovia Corp, 2020). Hydrogen bonds, hydrophobic interactions, and π-alkyl interactions were analyzed to understand binding stability.

RESULTS

Table 1 shows the binding affinities expressed in terms of the binding energy (kcal/mol), for each of the bioactive compound detected from CBEO using GC-MS analysis, against both chitin synthase and 1,3-beta-glucan synthase.

Table 1: Bioactive Compounds Detected in CBEO Using GC-MS Analysis

Peak #	Compound detected	PubChem CID	Retention time (min)	Peak Area %	Chitin synthase (kcal/mol)	Glucan synthase (kcal/mol)
1	beta-Phellandrene	11142	9.98	1.20	-5.462	-5.947
2	Linalool	6549	10.58	2.57	-4.913	-5.336
3	alpha-Terpineol	17100	14.75	0.63	-5.828	-6.223
4	Cinnamaldehyde, (E)	637511	15.26	36.56	-5.739	-5.471
5	Cinnamaldehyde,dimethylacetal	5463228	17.08	11.87	-6.046	-5.726
6	Eugenol	3314	17.50	4.60	-5.451	-5.548
7	Copaene	12303902	20.00	2.09	-6.692	-7.039
8	β-caryophyllene	5281515	20.75	1.46	-5.839	-7.657
9	Acetate,cinnamyl ester	5282110	21.75	1.17	-6.311	-6.096
10	Caryophyllenyl alcohol	91704770	23.00	3.36	-6.117	-7.686
11	Caryophylleneoxide	1742210	24.43	1.23	-5.808	-7.187
12	Tetradecanal	31291	25.50	1.19	-4.577	-4.846
13	BenzylBenzoate	2345	26.00	3.51	-6.937	-7.266

14	n-Hexadecanoic acid	985	27.70	2.48	-5.295	-5.514
15	Hexadecanoic acid, methyl ester	5325830	30.00	2.33	-5.694	-5.793
16	alpha.-Phellandrene,dimer	91747905	31.13	4.74	-	-8.211
17	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	6428995	32.41	4.84	-5.187	-5.286
18	Octadecenoic acid, methyl ester	7707	34.25	3.57	-5.364	-5.227

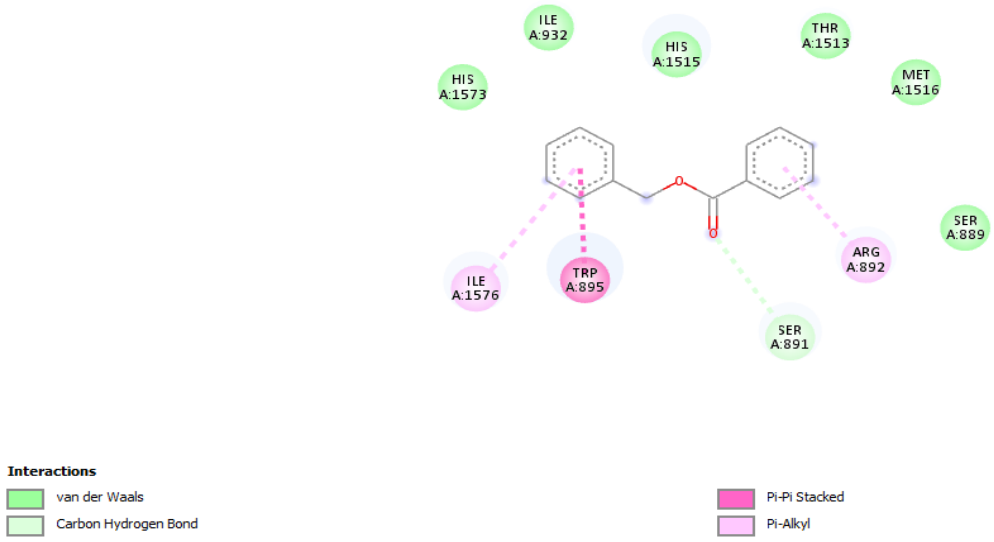
Table 2 shows the docking scores of five bioactive compounds from *Cinnamomum zeylanicum* showing the highest binding affinities against chitin synthase. Benzylbenzoate had the highest binding affinity with a docking score of −6.937 kcal/mol against the enzyme, which plays a key role in cell biosynthesis. Copaene followed with a docking score of −6.692 kcal/mol. Acetate, cinnamyl ester also showed a good docking score of −6.311 kcal/mol against the structural enzyme. Caryophyllenyl alcohol also showed a good binding affinity at a binding energy of -6.117 kcal/mol and lastly, the derivative of the major bioactive compound in the CBEO known as cinnamaldehyde, dimethylacetal with a docking score of -6.046 kcal/mol. The referral drug, nikkomycin Z, is included for comparison.

Table 2: Binding Affinities of CBEO Compounds to Chitin Synthase (CSH)

PubChem Cid	Compound Name	Binding energy (kcal/mol)
2345	BenzylBenzoate	-6.937
12303902	Copaene	-6.692
5282110	Acetate, cinnamyl ester	-6.311
91704770	Caryophyllenyl alcohol	-6.117
5463228	Cinnamaldehyde, dimethylacetal	-6.046
	Nikkomycin Z	-6.60

Figure 1a-c show the 2D- Visualization of the interactions between the protein-ligand complexes at the active site of CSH, involving various forces that influence and contribute to the stability of the complexes. Notable forces influencing these interactions include van der waals forces, pi-pi stacked, pi-alkyl bonds, pi-sigma bonds and conventional hydrogen bonds. Benzyl benzoate and copaene exhibited extensive hydrophobic interactions within the catalytic pocket, supporting their superior binding scores. These interactions suggest strong structural compatibility and potential interference with chitin polymerization.

(a)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ARG A:892
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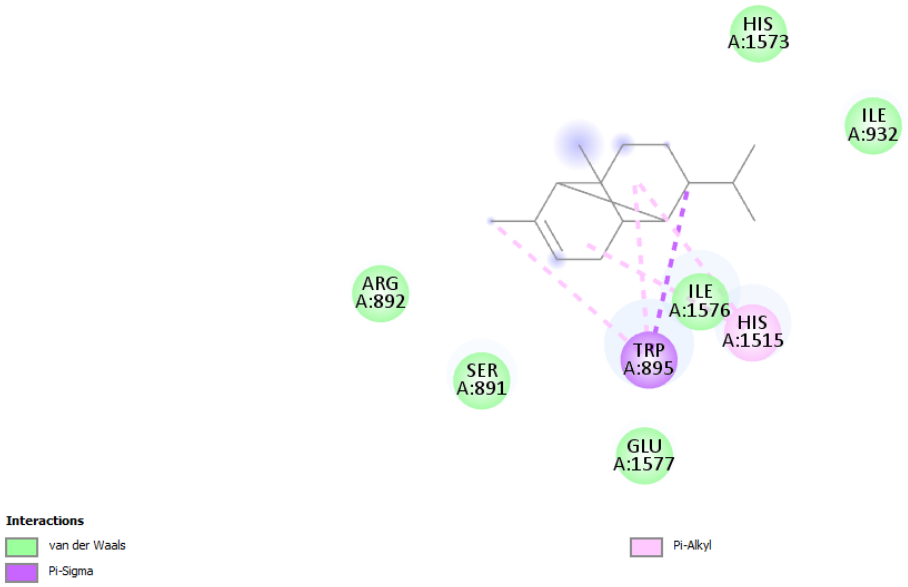
SER A:891
Interactions
van der Waals
Carbon Hydrogen Bond
Pi-Pi Stacked
Pi-Alkyl

(b)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ILE A:1576
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Interactions
van der Waals
Pi-Sigma
Pi-Alkyl

(c)

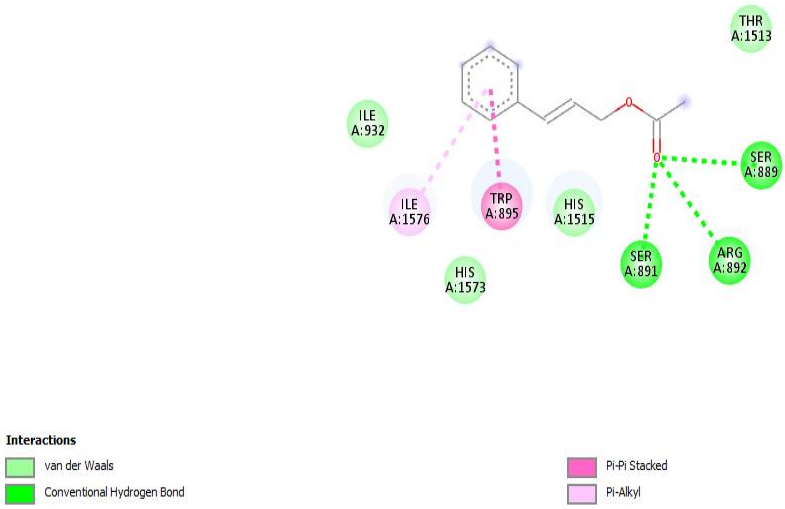


Figure 1. Two-dimensional (2D) visualization of the top-ranked *Cinnamomum zeylanicum* bark essential oil (CBEO) compounds docked within the active site of chitin synthase (CHS).
(a) BenzylBenzoate-CHS complex, (b) Copaene-CHS complex, (c) Acetate, cinnamyl ester-CHS

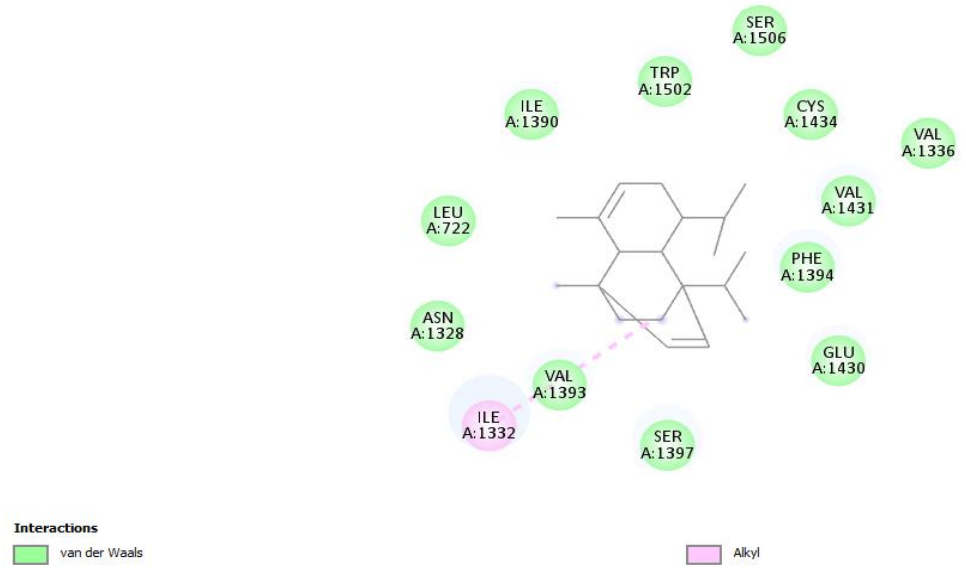
Table 3 shows the docking scores of five bioactive compound from CBEO having the highest binding affinities against 1,3-β-glucan synthase. Alpha.-phellandrene, dimer had the highest binding affinity with a docking score of −8.211 kcal/mol against the enzyme. Caryophyllenyl alcohol followed with a docking score of −7.686 kcal/mol. β-Caryophyllene also showed a good docking score of -7.657 kcal/mol against the structural enzyme. BenzylBenzoate also showed a good binding affinity at a binding energy of -7.266 kcal/mol and lastly, copaene with a docking score of -7.039 kcal/mol. The referral drug, caspofungin is included for comparison.

Table 3: Binding Affinities of CBEO Compounds to 1,3-beta-glucan Synthase

PubChem Cid	Compound Name	Binding energy (kcal/mol)
91747905	alpha.-Phellandrene, dimer	-8.211
91704770	Caryophyllenyl alcohol	-7.686
5281515	β-Caryophyllene	-7.657
2345	BenzylBenzoate	-7.266
12303902	Copaene	-7.039
-	Caspofungin	-5.30

Figure 2 a-c show the 2D- Visualization of the interactions between the protein-ligand complexes at the active site of 1,3-β-glucan synthase, involving various forces that influence and contribute to the stability of the complexes. Notable forces influencing these interactions include van der waals forces, pi-sulfur, pi-alkyl bonds, alkyl-interactions, pi-sigma bonds and carbon hydrogen bonds. The α-phellandrene dimer and sesquiterpene derivatives formed extensive hydrophobic contacts within the enzyme's catalytic region, consistent with their superior docking scores and structural complexity.

(a)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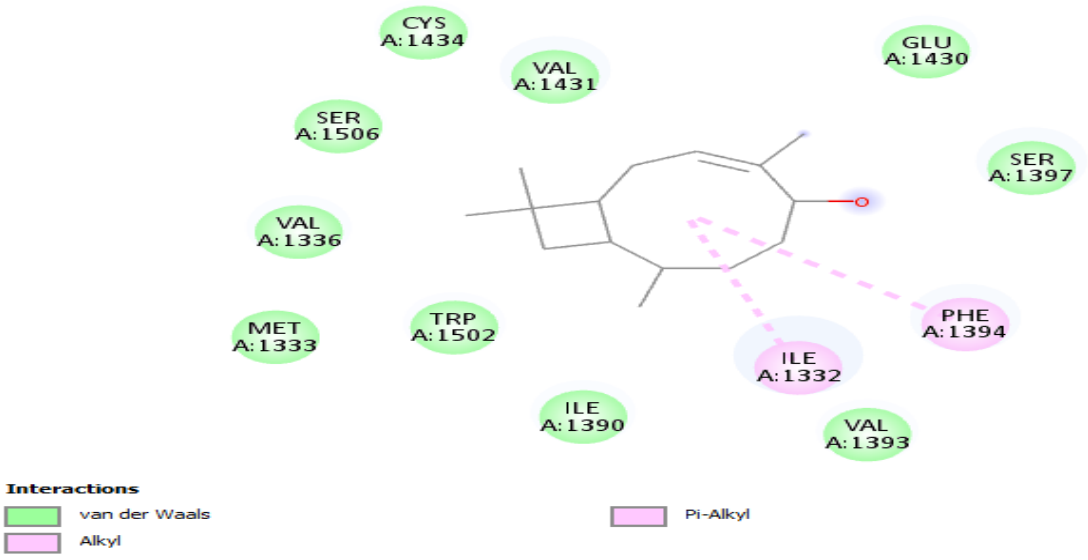
Interactions
van der Waals
Alkyl

(b)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Interactions
van der Waals
Alkyl
Pi-Alkyl

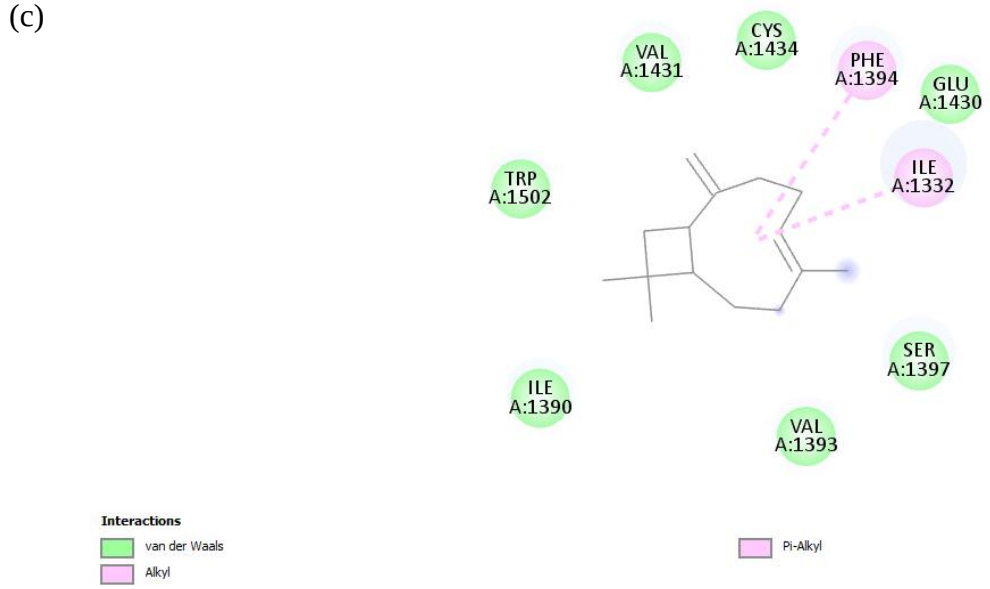

Figure 2. Two-dimensional (2D) visualization of the top-ranked CBEO compounds docked within the active site of 1,3-β-glucan synthase (FKS) (a) alpha-Phellandrene, dimer-FKS complex, (b) Caryophyllenyl alcohol-FKS complex, (c) β-caryophyllene-FKS complex.

DISCUSSION

The present *in silico* study provides mechanistic insight into the antifungal potential of *Cinnamomum zeylanicum* bark essential oil (CBEO) by evaluating its constituent compounds against two essential fungal cell-wall biosynthetic enzymes: chitin synthase (CHS) and 1,3-β-glucan synthase (FKS). These enzymes are indispensable for fungal growth, morphogenesis, and virulence, and their inhibition leads to cell-wall instability and fungal lysis (Lenardon *et al.*, 2010).

GC–MS profiling confirmed that CBEO is chemically diverse, with cinnamaldehyde as the dominant constituent. While cinnamaldehyde exhibited moderate binding affinities in the docking analysis, its established antifungal activity has been attributed primarily to membrane disruption, interference with ergosterol biosynthesis, and induction of oxidative stress rather than direct enzyme inhibition (Hyldgaard *et al.*, 2012; Zhang *et al.*, 2017). This observation supports the notion that essential oils exert antifungal effects through multiple complementary mechanisms, with minor constituents often playing critical roles in enzyme-level inhibition.

Docking analysis against chitin synthase revealed that benzyl benzoate, copaene, cinnamyl ester, caryophyllenyl alcohol, and cinnamaldehyde dimethyl acetal displayed binding affinities comparable to the reference inhibitor nikkomycin Z. Nikkomycin Z is a well-characterized competitive inhibitor of CHS that disrupts chitin polymerization, leading to weakened fungal cell walls (Walker *et al.*, 2015). The ability of CBEO constituents to approach the binding affinity of nikkomycin suggests potential inhibitory relevance. Interaction profiling further demonstrated that these ligands were stabilized primarily by hydrophobic contacts, π–alkyl interactions, and π–π stacking (Figure 1), which are critical determinants of ligand stability within CHS catalytic pockets (Lenardon *et al.*, 2010).

More pronounced inhibitory potential was observed against 1,3-β-glucan synthase, where several CBEO compounds exhibited binding affinities exceeding that of the clinically used

echinocandin caspofungin. Echinocandins inhibit glucan synthesis by targeting FKS subunits, resulting in osmotic fragility and fungal cell death (Perlin, 2015). In this study, the alpha-phellandrene dimer demonstrated the strongest affinity, followed by caryophyllenyl alcohol and β-caryophyllene. These sesquiterpene-rich compounds formed extensive hydrophobic and aromatic interactions within the FKS active site (Figure 2), consistent with previous reports highlighting the strong affinity of lipophilic terpenes for membrane-associated enzymes (Mutlu-Ingok *et al*., 2020).

The ability of certain CBEO constituents, particularly benzyl benzoate and copaene to interact favorably with both CHS and FKS suggests a dual-target inhibitory mechanism. Multi-target antifungal strategies are increasingly recognized as advantageous due to their reduced susceptibility to resistance development, a major limitation of single-target antifungal drugs (Fisher *et al*., 2018). Moreover, essential oil constituents are known to act synergistically, where combined effects exceed the activity of individual compounds (Hyldgaard *et al*., 2012).
Overall, this study aligns with existing literature supporting the antifungal potential of *Cinnamomum zeylanicum* derived compounds and extends current knowledge by providing molecular-level evidence for their interaction with validated fungal cell-wall targets.

CONCLUSION

The study demonstrated that *Cinnamomum zeylanicum* bark essential oil contains multiple bioactive constituents with strong *in silico* inhibitory potential against fungal chitin synthase and 1,3-β-glucan synthase. Benzyl benzoate, copaene, caryophyllenyl alcohol, and alpha-phellandrene dimer emerged as key candidates, exhibiting binding affinities comparable to or exceeding standard antifungal drugs. The results support the hypothesis that CBEO exerts antifungal activity through multi-component, multi-target mechanisms involving fungal cell-wall biosynthesis. These findings provide a robust computational foundation for further *in vitro* and *in vivo* validation and highlight CBEO as a promising source of natural antifungal agents for controlling mycotoxigenic fungi.

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