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Research Article

IN SILICO EVALUATION OF ANTIFUNGAL ACTIVITY OF SELECTED MEDICINAL PLANT DERIVED COMPOUNDS FOR CONTROLLING FUNGAL CONTAMINATION IN LIVESTOCK FEED

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ABSTRACT

In silico molecular docking studies using AutoDock Vina were conducted to evaluate the antifungal potential of five plant derived bioactive compounds azadirachtin, emodin, curcumin, aloin and eugenol against therapeutically relevant enzymes from toxigenic fungi proteins, NADase from *Aspergillus fumigatus* (PDB 6YGF), FADGDH from *Aspergillus flavus* (PDB 4YNU), glucose oxidase from *Aspergillus niger* (PDB 1GAL) and sfPAFB from *Penicillium chrysogenum* (PDB 2NC2). Azadirachtin exhibited the highest binding affinities (-17.0 to -9.7 kcal/mol), forming favourable interactions at conserved catalytic sites including His505/His548 in FADGDH, outperforming comparator compounds. Swiss ADME pharmacokinetic profiling confirmed drug-likeness compliance for emodin, curcumin, and eugenol (bioavailability score 0.55), whereas azadirachtin showed violations attributable to elevated molecular weight (720.71 g/mol) and reduced score (0.17). These results identify azadirachtin as a promising lead candidate for pharmaceutical development targeting fungal contamination in livestock feed, necessitating experimental validation through *in vitro* and *in vivo* assays.

Keywords: Azadirachtin, Molecular docking, AutoDock Vina, SwissADME, Antifungal agents, Drug-likeness.

INTRODUCTION

The contamination of livestock feedstuffs by toxigenic fungi represents a major challenge to animal health, productivity, and overall food safety. Ensuring safe and uncontaminated feed is therefore essential for maintaining optimal animal health and performance (Yaswanthkumar *et al*., 2021). Filamentous fungi such as *Aspergillus*, *Penicillium*, *Fusarium* and *Alternaria spp.* are frequently implicated in feed spoilage and are well known for producing a wide range of mycotoxins, a secondary metabolite with potent toxicological effects (Smith *et al.*, 2008; Khalifa *et al*., 2022). These mycotoxins can contaminate feed ingredients at multiple stages, including pre-harvest, harvesting, processing and storage. These types of contaminations are commonly detected in cereal grains such as maize, wheat and barley as well as in oilseeds and their by-products (EFSA, 2008). Exposure to mycotoxin contaminated feed can impair feed intake, immune competence and gastrointestinal integrity ultimately reducing growth performance and productivity of the animals. Chronic exposure has been associated with hepatotoxicity, nephrotoxicity, reproductive abnormalities and increased susceptibility to infectious diseases with severe contamination occasionally resulting in mortality (Khalif *et al*., 2022; Guluwa *et al*., 2023). Mitigation strategies for managing fungal contamination in feed typically involve improving storage and processing conditions, routine monitoring for fungal load and incorporating mycotoxin binders or detoxifying agents into feed formulations (EFSA, 2008). Additionally, agronomic interventions such as cultivating fungal resistant crop varieties and implementing good agricultural practices serve as preventive measures to reduce mycotoxin occurrence at the production level (Guluwa *et al*., 2023). In recent years, bioactive compounds derived from plants and

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microorganisms have gained significant attention as natural antifungal and detoxifying agents. Polyphenols, terpenes and other phytochemicals sourced from agri-food by-products including grape marc, grape seeds and pear extracts have demonstrated promising fungicidal activity against mycotoxigenic fungi such as *Aspergillus* and *Fusarium spp.* (Sharma and Salunke, 2021). These compounds have also been reported to reduce levels of key mycotoxins, including aflatoxins, ochratoxin A and deoxynivalenol[7]. Microbial and enzymatic detoxification strategies, offering biodegradable and environmentally friendly alternatives to synthetic fungicides, further highlight the potential of biotechnological interventions (Ates *et al*., 2020; Grenier *et al*., 2011). Advances in computational biology have expanded opportunities for identifying novel antifungal agents with high precision and efficiency (Anderson *et al*., 2023). The modern techniques like, in-silico approaches particularly molecular docking and virtual screening enables the rapid prediction of binding affinities, inhibitory potential and molecular interactions between bioactive compounds and key fungal targets (Anderson *et al*., 2023). These tools facilitate the rational design and selection of promising antifungal candidates by allowing the assessment of structural compatibility with fungal enzymes and receptors essential for survival and pathogenicity (Patil *et al*., 2017). By integrating molecular docking and computational analysis to identify the plant-derived compounds which are capable of mitigating fungal contamination in stored feedstuffs and to develop natural, safe and effective antifungal interventions for safeguarding animal health and maintaining feed quality (Patil *et al*., 2017). The present study employs in silico methodologies to evaluate selected bioactive compounds from easy and locally available medicinal plants for their potential antifungal activity against commonly encountered feed borne fungi.

MATERIALS AND METHODS

Protein Structure Retrieval and Preparation

The three-dimensional structures of target fungal enzymes were obtained from the Research Collaboratory for Structural Bioinformatics Protein Data Bank (RCSB PDB). The selected proteins included NADase from *Aspergillus fumigatus* (Figure1), FAD-dependent glucose dehydrogenase (FADGDH) from *A. flavus* (Figure 2), glucose oxidase from *A. niger* (Figure 3) and the antifungal protein sfPAFB from *Penicillium chrysogenum* (Figure 4) (Table 1). Structures were selected based on resolution quality, structural completeness, and absence of mutations.

Table 1. Targeted protein of selected fungi.

PDB ID	Protein	Organism	Resolution (Å)	Method	UniProt ID
6YGF	NADase	*A. fumigatus*	1.6	X-ray	Q4WL81
4YNU	FADGDH	*A. flavus*	1.5	X-ray	-
1GAL	GOx	*A. niger*	2.3	X-ray	P13006
2NC2	sfPAFB	*P. chrysogenum*	NMR	NMR	-

Protein preparation was performed by using BIOVIA Discovery Studio 2024 and AutoDockTools v1.5.7. The downloaded PDB files were analyzed for co-crystallized ligands, ions, and heteroatoms. All crystallographic water molecules and non-essential heteroatoms were removed, except essential cofactors such as FAD in FADGDH (PDB 4YNU) and glucose oxidase (PDB 1GAL). Polar hydrogens were added, non-polar hydrogens merged and Gasteiger partial charges were assigned to the protein atoms. Kollman united-atom charges were uniformly applied. Catalytic and ligand-binding residues were identified from primary literature and docking grid boxes were centered on these functional sites, with dimensions set to 25 × 25 × 25 Å and a spacing of 0.375 Å. An exhaustiveness level of 8 was used to ensure optimal conformational sampling. Final prepared receptors were saved in PDBQT format for docking simulations.

Ligand Preparation

Selected ligands were retrieved as three-dimensional structures from the PubChem database (Figure 5,6,7,8 and 9) (Kim *et al.*, 2016). The compounds energy were minimized, then converted into dock able PDBQT format using PyRx 0.8 software, implementing AutoDock Vina protocols for assigning proper partial charges and torsional flexibility (Table 2).

Table 2. Selected ligand to study anti-fungal properties.

CID	Compound Name	Source Plant	Molecular Formula	MW (g/mol)
5281303	Azadirachtin	*Azadirachta indica*	$C_{35}H_{44}O_{16}$	720.71
3220	Emodin	*Rheum spp.*	$C_{15}H_{10}O_5$	270.24
969516	Curcumin	*Curcuma longa*	$C_{21}H_{20}O_6$	368.38
12305761	Aloin	*Aloe vera*	$C_{21}H_{22}O_9$	418.39
3314	Eugenol	*Syzygium aromaticum*	$C_{10}H_{12}O_2$	164.20

Molecular Docking

The antifungal potential of the bioactive compounds was evaluated through molecular docking, an established in silico technique for virtual screening to predict ligand target interactions and binding affinities (Kontoyianni, 2017). Docking simulations were conducted using PyRx, an open-source platform designed for efficient docking of small molecule libraries to macromolecules, facilitating identification of lead compounds with potential biological activity (Dallakyan and Olson, 2015).

Molecular Docking Process

Prior to docking, the macromolecular structures were refined using BIOVIA Discovery Studio 2024 correcting bond geometries, removing extraneous chemical moieties and water molecules, adding hydrogen atoms, and saving in PDBQT format. Subsequently, the prepared 3D protein structures were docked against the selected bioactive ligands using PyRx software under standardized parameters.

Visualization and Molecular Interaction Analysis

Top-ranked docking poses were selected based on lowest binding free energies (ΔG bind), were visualized and analyzed using BIOVIA Discovery Studio Visualizer 2016. This molecular modelling tool allows detailed examination of protein-ligand interactions, aiding in the interpretation of key binding residues and interaction types (Biovia DS, 2017).

Drug-Likeness Features Evaluation and ADME Evaluation

The Swiss ADME server can be used to analyse the pharmacokinetic profiles of the selected bioactive compounds. Swiss ADME was used to identify each compound's drug-like properties. The physicochemical properties of small-molecule drugs significantly impact their permeability, primarily via passive diffusion. Early detection of these features is crucial for successful drug discovery research because it eliminates molecules with undesirable pharmacokinetic properties can help forecast performance in a real-world setting (Daina *et al*., 2017). Following Lipinski's "rule of five (Lipinski *et al*., 2001)," molecules should have a molecular weight (MW) below 500, several hydrogen bond donors (HBD) below 5, several hydrogen bond acceptors (HBA) below 10, and a consensus log p-value (log p) below five (Noe and Peakman, 2017) the better bioactive compounds were suggested to taken for the further analysis.

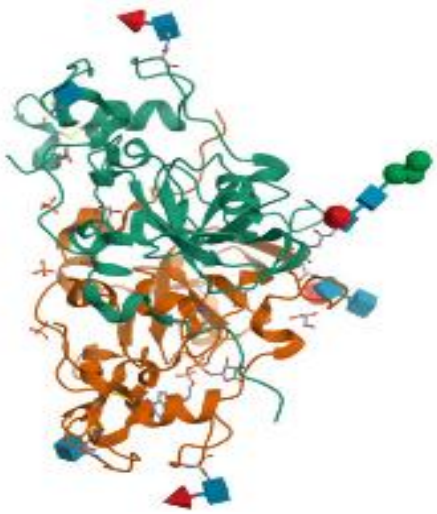


Figure 1. NADase from *Aspergillus fumigatus* (PDB id. 6YGF)

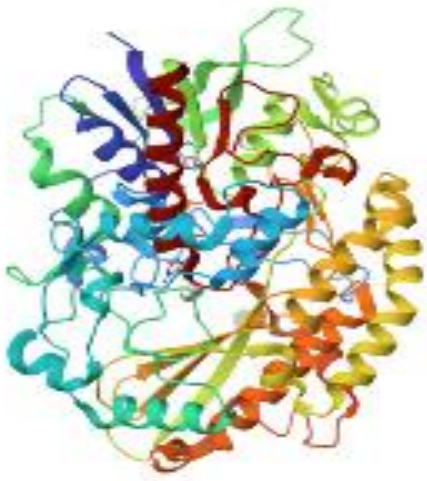


Figure 2. FAD-dependent glucose dehydrogenase (FADGDH) from *A. flavus* (PDB id. 4YNU)

Figure 3. Glucose oxidase from *A. niger* (PDB id. 1GAL)

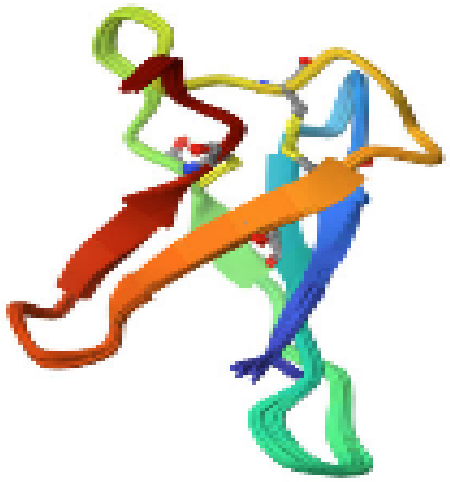


Figure 4. sfPAFB from *Penicillium chrysogenum* (PDB id. 2NC2)

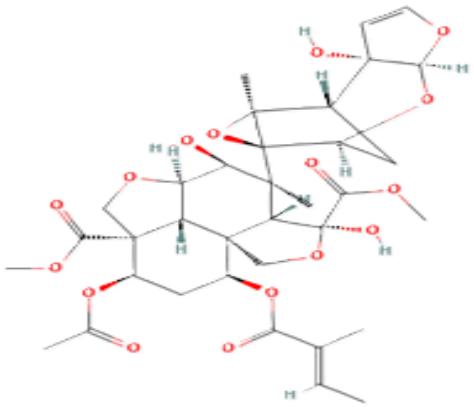


Figure 5. Azadirachtin (Pub chem ID:5281303**)**

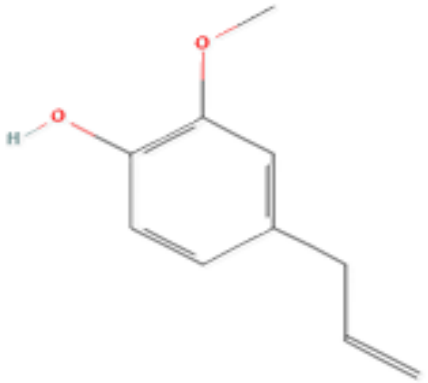


Figure 6. Emodin (Pub chem ID:3220)

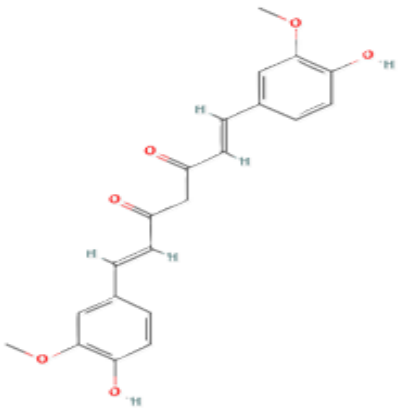


Figure 7. Curcumin (Pub chem ID:969516)

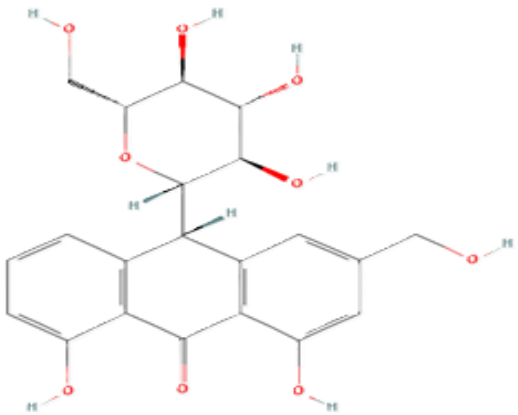


Figure 8. Aloin (Pub chem ID:12305761)

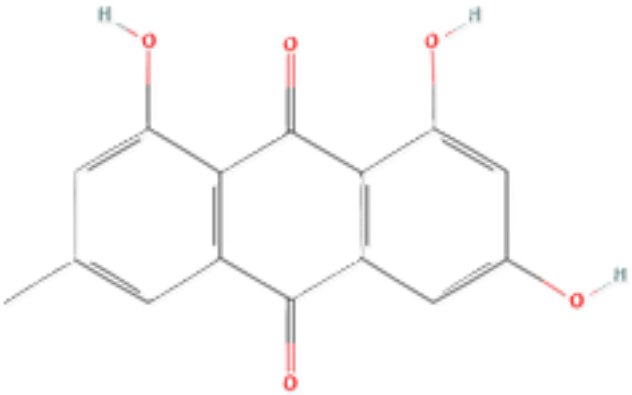


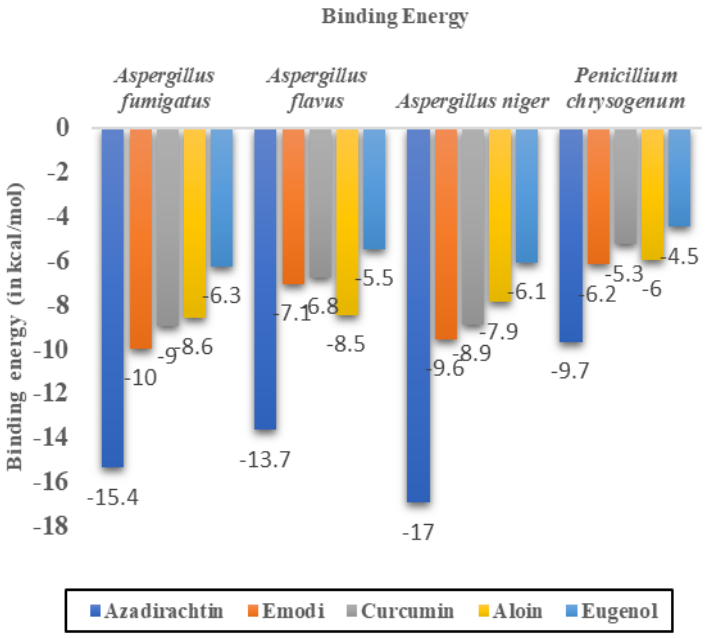
Figure 9. Eugenol (Pub chem ID:3314).

RESULTS AND DISCUSSION

The binding affinities (ΔG bind, kcal/mol) of the five bioactive compounds against target fungal enzymes were summarized in Table 3(Graph.1). Azadirachtin consistently exhibited the highest affinities across all targets, ranging from -9.7 to -17.0 kcal/mol, with peak potency against glucose oxidase from *A. niger* (PDB 1GAL; -17.0 kcal/mol). Azadirachtin demonstrated superior binding to NADase (*A. fumigatus*; -15.4 kcal/mol) and FADGDH (*A. flavus*; -13.7 kcal/mol) compared to other ligands. Emodin showed strong interactions with NADase (-10.0 kcal/mol) and glucose oxidase (-9.6 kcal/mol), while aloin exhibited competitive binding to FADGDH (-8.5 kcal/mol). Curcumin and eugenol displayed moderate to weak affinities across targets, with eugenol consistently lowest (-4.5 to -6.3 kcal/mol). Three-dimensional binding poses and corresponding 2D interaction diagrams were shown for (a) azadirachtin, emodin, curcumin, aloin, and eugenol in the active sites of the four fungal proteins. NADase from *Aspergillus fumigatus* (PDB 6YGF) illustrate ligand orientation within the catalytic pocket, with the protein displayed as a cartoon and ligands as sticks, highlighting hydrogen bonds, hydrophobic contacts, and other stabilizing interactions (Figure 10). FAD-dependent glucose dehydrogenase (FADGDH) from *A. flavus* depict ligand binding relative to the catalytic residues and FAD cofactor, with key hydrogen-bonding and hydrophobic interactions annotated in the 2D maps (Figure 11). Panels for glucose oxidase from *A. niger* show the spatial fit of each ligand in the substrate-binding cavity and the network of hydrogen bonds, π–π stacking and hydrophobic contacts formed with surrounding residues (Figure 12). The antifungal protein sfPAFB from *Penicillium chrysogenum* display ligand orientation in the binding pocket and corresponding 2D interaction maps, emphasizing the amino acid residues contributing to complex stabilization and supporting the predicted antifungal activity of the tested compounds (Figure 13).

Table 3. The table lists five compounds: Azadirachtin, Emodin, Curcumin, Aloin, and Eugenol, along with their respective PubChem IDs.

S.No.	Pub chem ID	Compound name	Binding affinity (in kcal/mol) *A. fumigatus* (6YGF)	*A. flavus* (4YNU)	*A. niger* (1GAL)	*P. chrysogenum* (2NC2)
1	5281303	Azadirachtin	-15.4	-13.7	-17	-9.7
2	3220	Emodi	-10	-7.1	-9.6	-6.2
3	969516	Curcumin	-9	-6.8	-8.9	-5.3
4	12305761	Aloin	-8.6	-8.5	-7.9	-6
5	3314	Eugenol	-6.3	-5.5	-6.1	-4.5



Graph 1. Binding energy of Azadirachtin, Emodin, Curcumin, Aloin, and Eugenol with *Aspergillus fumigatus*, *Aspergillus flavus, Aspergillus niger* and *Penicillium chrysogenum.*

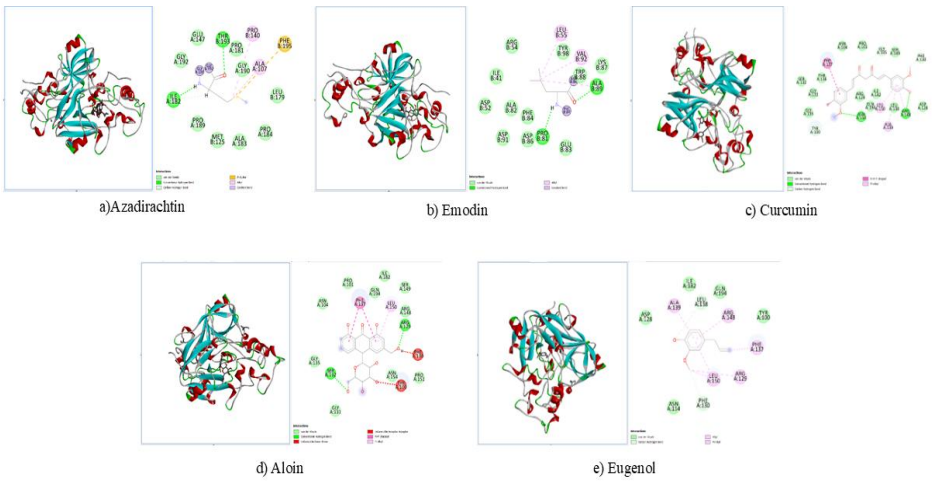


Figure 10. Ligand interactions with NADase from *Aspergillus fumigatus* (PDB 6YGF).

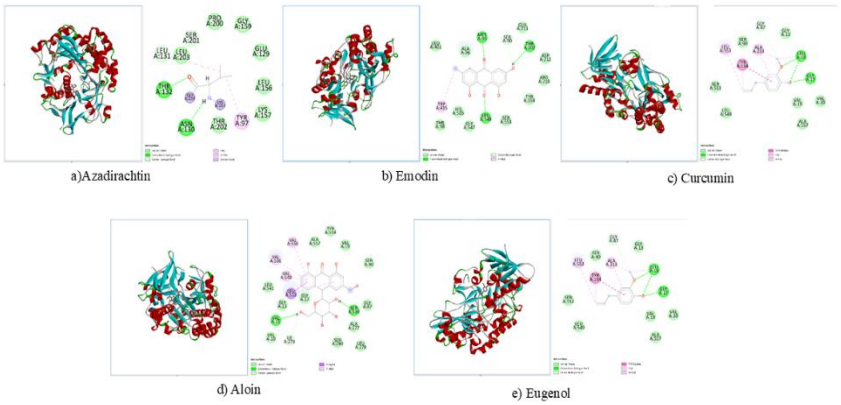


Figure 11. Ligand interactions with FADGDH from *Aspergillus flavus* (PDB 4YNU).

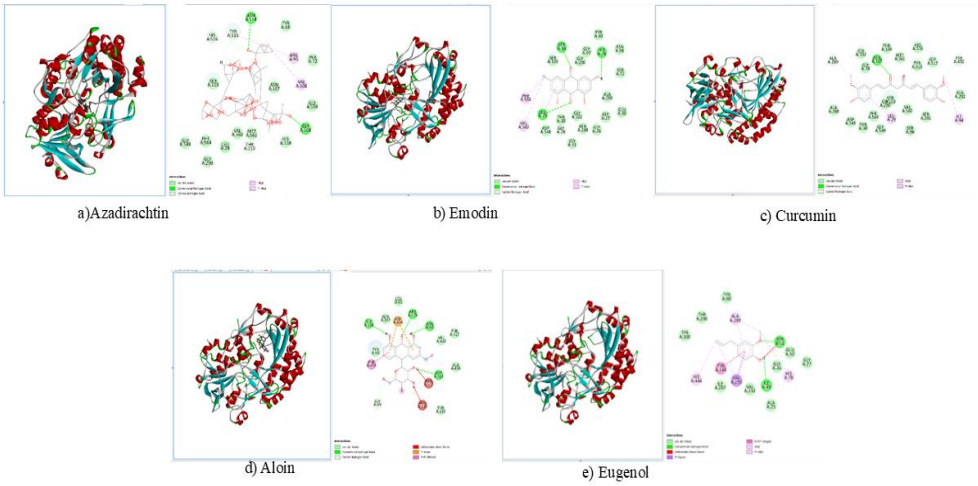


Figure 12. Ligand interactions with glucose oxidase from *Aspergillus niger* (PDB 1GAL).

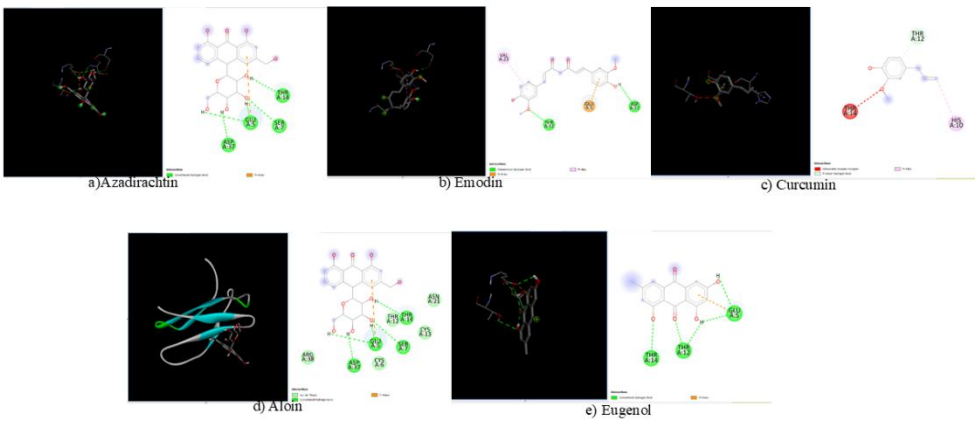


Figure 13. Ligand interactions with sfPAFB from *Penicillium chrysogenum*.

The pharmacokinetic properties and drug-likeness of selected bioactive compounds were comprehensively analyzed to assess their potential as antifungal agents (Table 4). Azadirachtin ($C_{35}H_{44}O_{16}$) has a molecular weight of 720.71 g/mol and a Topological Polar Surface Area (TPSA) of 215.34 Å², with moderate lipophilicity indicated by a consensus Log P o/w of 0.88. It is moderately soluble in water but exhibits low gastrointestinal absorption and does not permeate the blood-brain barrier (BBB). Azadirachtin is a substrate for P-glycoprotein (P-gp) but does not inhibit major cytochrome P450 enzymes (CYP1A2, CYP2C19, CYP2C9, CYP2D6, CYP3A4). It violates multiple drug-likeness filters including Lipinski, Ghose, Veber, Egan, and Muegge—primarily due to high molecular weight and TPSA. Despite these violations, no PAINS alerts were detected, and only minor Brenk alerts were present. The bioavailability score is low (0.17), and the synthetic accessibility score is high (8.11), indicating challenges for synthesis and formulation. Emodin ($C_{15}H_{10}O_5$) has a molecular weight of 270.24 g/mol and TPSA of 94.83 Å², with moderate lipophilicity (Log P o/w 1.87). It is water-soluble, shows high gastrointestinal absorption, but does not cross the BBB. Emodin is not a P-gp substrate but inhibits CYP1A2 and CYP3A4 enzymes. It complies with all major drug-likeness rules with no violations, although one PAINS alert (quinone A) was noted. Bioavailability is moderate (0.55), with moderate synthetic accessibility (2.57). Curcumin ($C_{21}H_{20}O_6$) has molecular weight 368.38 g/mol and TPSA of 93.08 Å², with moderate lipophilicity (Log P o/w 3.03). It is soluble, has high gastrointestinal absorption, and does not permeate the BBB. Curcumin is not a P-gp substrate and inhibits CYP2C9 and CYP3A4 but not CYP1A2, CYP2C19, or CYP2D6. It satisfies all drug-likeness criteria with no violations but presents two Brenk alerts (beta_keto_anhydride, michael_acceptor_1). Bioavailability score is 0.55, and synthetic accessibility is moderate (2.97). Aloin ($C_{21}H_{22}O_9$) displays a molecular weight of 418.39 g/mol and TPSA of 181.62 Å², with moderate lipophilicity (Log P o/w 3.03). It is moderately soluble, has high gastrointestinal absorption, and does not cross the BBB. Aloin is not a P-gp substrate and inhibits CYP2C19, CYP2C9, and CYP3A4 enzymes. It complies with all drug-likeness rules without violations and shows two Brenk alerts identical to curcumin. Bioavailability and synthetic accessibility scores are 0.55 and 2.97, respectively. Eugenol ($C_{10}H_{12}O_2$) has low molecular weight (164.20 g/mol) and low TPSA (29.46 Å²), with moderate lipophilicity (Log P o/w 2.25). It is water-soluble, with high gastrointestinal absorption and is permeable to the BBB. Eugenol is not a P-gp substrate but inhibits CYP1A2. It exhibits full compliance with drug-likeness filters and presents one Brenk alert (isolated alkene). Bioavailability score is 0.55, with low synthetic accessibility score (1.58), suggesting ease of synthesis.

Table 4. ADME and drug-likeness properties of bioactive compounds (Swiss ADME).

Property	Azadirachtin	Emodin	Curcumin	Aloin	Eugenol
MW (g/mol)	720.71	270.24	368.38	418.39	164.20
TPSA (Å²)	215.34	94.83	93.08	181.62	29.46
LogP_o/w	0.88	1.87	3.03	3.03	2.25
Solubility	Moderately	Soluble	Soluble	Moderately	Soluble
GI Absorption	Low	High	High	High	High
BBB Permeant	No	No	No	No	Yes
P-gp Substrate	Yes	No	No	No	No
CYP Inhibition	None	1A2,3A4	2C9,3A4	2C19,2C9,3A4	1A2
Lipinski Violations	3	0	0	0	0
PAINS Alerts	0	1	0	0	0
Brenk Alerts	Few	0	2	2	1
Bioavailability	0.17	0.55	0.55	0.55	0.55
Synth. Accessibility	8.11	2.57	2.97	2.97	1.58

The fungal contamination in livestock feed primarily involves toxigenic moulds like Aspergillus, Penicillium and Rhizopus which produce mycotoxins such as aflatoxins that threaten animal health and productivity (Rahimi and Rahimi, 2017). Studies show high contamination rates in cattle feed components: up to 55% of rations by *Aspergillus* (Khalifa *et al*., 2022). The molecular docking results for the five compounds (Azadirachtin, Emodi, Curcumin, Aloin, and Eugenol) against the four fungi (*Aspergillus fumigatus*, A*spergillus flavus*, *Aspergillus niger*, and *Penicillium chrysogenum*) revealed significant insights. The binding affinities (in kcal/mol) for each compound against the four fungi were shows in negative values. Negative values indicate stronger binding affinities, with more negative values suggesting stronger interactions (Morris and Lim wilby, 2008; Ferreira *et al*., 2015). Binding affinity is a crucial concept in molecular docking and drug discovery, as it quantifies the strength of interaction between a ligand (such as a drug or compound) and its target protein. Lower (more negative) binding affinity values indicate stronger interactions, suggesting that the ligand is more likely to bind effectively to the target (Lionta *et al*., 2015). This

predictive value is significant in assessing the potential efficacy of a compound, particularly in antifungal activity. Compounds with strong binding affinities to key fungal proteins are more likely to inhibit fungal growth effectively. Understanding binding affinity also aids in optimizing drug candidates by allowing researchers to identify the most promising molecules for further development. Moreover, it provides insights into the mechanism of action of a compound at the molecular level, guiding experimental validation through in vitro and in vivo studies. Finally, high binding affinity values obtained from molecular docking studies can guide experimental validation, saving time and resources in the drug development process (Sliwoski et al., 2014). This comprehensive understanding of binding affinity's role in drug discovery is supported by various studies (Voet *et al*., 2016). In this study, Azadirachtin exhibited the strongest binding affinities across all tested fungi, suggesting its potential as a highly effective antifungal agent (Abd-Elhalim *et al*., 2025). Emodi and Curcumin also showed significant binding affinities, particularly against (Rathore. 2021). Aloin and Eugenol exhibited moderate binding affinities, suggesting they may still be effective but perhaps less potent compared to Azadirachtin (Zhang *et al*., 2024).

Azadirachtin appears to be the most promising compound for use as an antifungal agent in livestock feed affecting fungi. Azadirachtin, derived from the neem tree (*Azadirachta indica*), has shown significant antifungal properties. It is known for its broad-spectrum antimicrobial activity, including antifungal effects, and disrupts the growth and development of fungi, making it an effective antifungal agent. Studies have demonstrated its efficacy against various fungal pathogens, including those affecting livestock feed. Azadirachtin is also considered an eco-friendly alternative to synthetic antifungal agents, as it is biodegradable and has low toxicity to non-target organisms, making it safe for use in livestock feed (Ray, 2022; Mohideen *et al*., 2022; Singh *et al*., 2025). While Emodin, Curcumin, Aloin, and Eugenol also demonstrate antifungal properties, their effectiveness against livestock feed fungi is less documented. Emodin, found in various plants including aloe vera, has shown antifungal activity but requires further investigation for its efficacy in livestock feed (Patil *et al*., 2017). Curcumin, derived from turmeric, has been recognized for its antimicrobial properties, including antifungal activity. It has potential as a feed additive for livestock, with documented health benefits, but its specific antifungal efficacy in livestock feed needs more research (Sureshbabu *et al*., 2023; GreenSky Bio, 2024; Anas *et al*., 2024). Aloin, also found in aloe vera, has demonstrated antifungal properties, but its effectiveness against livestock feed fungi is less documented compared to Azadirachtin (Yadav, 2017; Vinay. 2021). Eugenol, a compound found in clove oil, has shown antifungal activity and potential as a feed additive for livestock, but its specific antifungal efficacy in livestock feed requires further research (Mak *et al*., 2019; Widodo and Hafsah, 2019).

CONCLUSION

This study demonstrates that binding affinity derived from molecular docking is a useful predictive indicator of antifungal potential, supporting the prioritization and optimization of plant-derived bioactive compounds as lead candidates. The docking and ADME analyses provide initial structure activity relationship insights, clarifying how specific interactions at fungal protein active sites may underlie the observed antifungal effects. Comparative evaluation of these compounds against known antifungal agents can further delineate their relative advantages and limitations as prospective therapeutics. Nevertheless, docking-based predictions remain hypothesis-generating and rigorous in vitro and in vivo studies are required to validate antifungal efficacy, refine dose regimens and assess safety in the context of livestock feed applications.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest

ETHICS APPROVAL

Not applicable

FUNDING

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AI TOOL DECLARATION

The authors declares that no AI and related tools are used to write the scientific content of this manuscript.

DATA AVAILABILITY

Data will be available on request

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