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Molecular Docking Analysis of Potential Fatty Acid Compounds from Tiger Milk Mushroom (*Lignosus rhinocerus* (Cooke) Ryvarden) Against Thioesterase Domain of Polyketide Synthase Enzyme in *Aspergillus* ssp.

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ABSTRACT

Fungal growth, particularly from species like *Aspergillus*, poses significant economic, agricultural, and health risks to humans due to aflatoxin production. Consequently, inhibiting aflatoxin synthesis has become a critical objective. In this study, researchers targeted the thioesterase (TE) domain of the Polyketide synthase enzyme for in silico docking experiments using AutoDock Vina. The aim was to identify potential inhibitors that could selectively target the TE domain. Various fatty acids from Lignosus rhinocerus were employed for this purpose, including lauric acid, decanoic acid, tetradecanoic acid-methyl ester, hexadecanoic acid, propionic acid, palmitic acid, stearic acid, and oleic acid. Decanoic acid showed promising results with binding energy close to the standard, forming two conventional hydrogen bonds, and exhibiting hydrophobic interactions during docking with the 3ILS protein. These findings suggest that decanoic acid could be utilized for inhibiting and controlling aflatoxin contamination in agricultural crops.
Keyword: Aflatoxin, In Silico Docking, *Lignosus rhinocerus*, Thioesterase Domain

ABSTRAK

Pertumbuhan jamur, terutama dari spesies seperti *Aspergillus*, menyebabkan kerugian di bidang ekonomi, pertanian, dan kesehatan yang signifikan bagi manusia karena produksi aflatoksin. Oleh karena itu, penting untuk menghambat sintesis aflatoksin. Dalam penelitian ini, peneliti menargetkan domain thioesterase (TE) dari enzim poliketida sintase untuk eksperimen docking in silico menggunakan AutoDock Vina. Tujuannya adalah untuk mengidentifikasi inhibitor potensial yang dapat secara selektif menargetkan domain TE. Berbagai asam lemak dari *Lignosus rhinocerus* digunakan untuk tujuan ini, yaitu asam laurat, asam dekanoat, asam tetradekanoat,-metil ester asam heksadekanoat, asam propionat, asam palmitat, asam stearat, dan asam oleat. Asam dekanoat menunjukkan hasil yang menjanjikan dengan energi pengikatan yang mendekati standar, membentuk dua ikatan hidrogen konvensional, dan menunjukkan interaksi hidrofobik selama docking dengan protein 3ILS. Temuan ini mengartikan bahwa asam dekanoat dapat digunakan untuk menghambat dan mengendalikan kontaminasi aflatoxin pada tanaman pertanian.
Keyword: Aflatoksin, Docking In Silico, *Lignosus rhinocerus*, Domain Thioesterase.

1. Introduction

Food contamination with aflatoxin can be a severe issue that could have a major negative impact on consumers' health. Several species of *Aspergillus* fungi, including *Aspergillus flavus* and *Aspergillus parasiticus*, produce aflatoxin, a toxic substance. Aflatoxin type B, which targets the liver and can result in

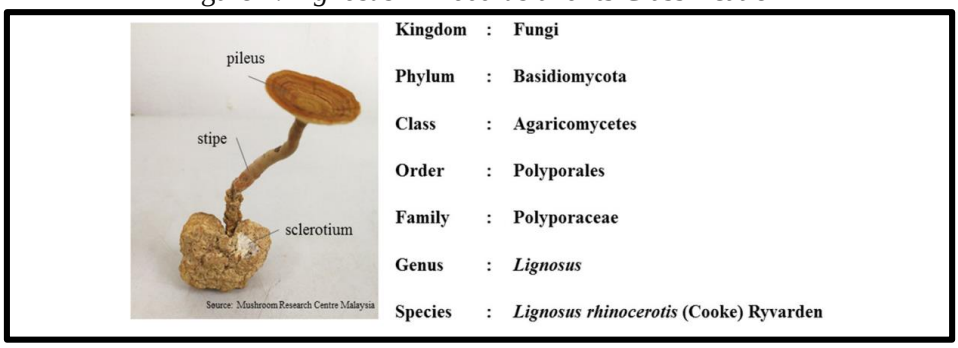
acute liver failure and death at high exposure levels, is the most dangerous aflatoxin [1]. There are beneficial fungi with high nutritional value that can be eaten in addition to harmful ones. Fatty acids are among the nutrients found in helpful fungi. Fats serve a variety of purposes in the body, including energy production, organ protection, insulators in the regulation of body temperature, mediators of biological activity between cells, and solvents for the vitamins A, D, E, and K. Fatty acids and glycerol, which are produced through the hydrolysis of fats, oils, and other lipid compounds, make up the fundamental building blocks of fats [2]

The fungus *Lignosus rhinocerus*, commonly referred to as Tiger Milk Mushroom (Figure 1), is capable of producing bioactive substances, such as fatty acids. Because of this mushroom's potential health benefits, it has long been used in traditional medicine in several Southeast Asian countries. Fatty acids are among *Lignosus rhinocerus*'s intriguing ingredients. *Lignosus rhinocerus* also has a high concentration of important fatty acids. There have been previous descriptions of the significance of these fatty acids in human metabolism. Certain fatty acids are thought to be unnecessary, but they play crucial physiological roles [3]. Oleic acid, for instance, lowers cholesterol and lowers the risk of cardiovascular illnesses. Niacin and riboflavin, which are crucial for human metabolism, were abundant in *Lignosus rhinocerus* samples, particularly the mycelium [4].

The enzyme polyketide synthase (PKS) is an essential part of the aflatoxin cluster, which is in charge of producing aflatoxin B1, a carcinogenic secondary metabolite [5]. A crucial stage in the biosynthesis of fungal polyketide products is the release of the final polyketide intermediate from refined polyketide synthases (iPKS), which is frequently made possible by the thioesterase (TE) domain. For interactions with different polyketide intermediates, the TE domain provides a flexible pathway that facilitates the formation of pyrones, hydrolysis, transesterification, and macrocyclization [6][7]. Controlling the production and contamination of aflatoxin can be achieved by inhibiting these enzymes that are specific to fungal polyketide synthesis [8].

The ability of *Lignosus rhinocerus* to create advantageous fatty acids is gaining interest as a potential anti-aflatoxin agent, despite the fact that research on this mushroom's usefulness in lowering aflatoxin contamination is currently limited. *Lignosus rhinocerus* produces fatty acids that may have antifungal qualities that can stop the development of fungi that create aflatoxin or even lessen the amount of aflatoxin that is produced. The potential of the fatty acids generated by Lignosus rhinocerus as anti-aflatoxin will be investigated in this report.

Figure 1. *Lignosus rhinocerus* and its Classification



(Source: Mushroom Research Centre Malaysia)

2. Method

2.1 Material

The structures of fatty acid compounds from tiger milk mushroom and curcumin as a standard were obtained from the PubChem Database (https://pubchem.ncbi.nlm.nih.gov/) [9]. Meanwhile, the aflatoxin biosynthesis polyketide synthase protein (PDB ID: 3ILS) was retrieved from the Protein Data Bank (https://www.rcsb.org/) [10].

2.2 Extraction of Mushroom Sclerotia

The extraction process followed the methodology described by Sari [11]. Initially, 500 grams of tiger milk mushroom sclerotia, obtained from local farmers, were cleaned and dried. The sclerotia were then pulverized into a powder using a blender. This powder was subjected to extraction by maceration in 96% ethanol solvent for 4×24 hours at room temperature, utilizing a shaker operating at 150 rpm. Afterward, the resultant mixture underwent filtration to separate the residue from the filtrate, which was then concentrated using a rotary evaporator until a thick extract was formed. Lastly, the extract was diluted to a 50%

concentration using 5% Dimethyl Sulfoxide (DMSO) solvent.

2.3 Detection of Fatty Acid by GC-MS Technique

The GC-MS QP-2010 (Shimadzu, Germany) with an RTX-5MS column (30×0.25×0.10m) was used to identify fatty acids. The oven temperature was programmed to increase from 60 °C to 220 °C over 30 minutes, with a ramp rate of 5 °C per minute, using a 1 µl injection volume. A 60 kPa pressure was used, and the overall flow rate was set at 37.5 ml/min. The carrier gas was helium, which had a linear velocity of 37.1 cm/s and a flow rate of 1.03 ml/min. Splitless mode was used to inject samples, and the scanning range was adjusted to 30 to 600 (m/z). Utilizing the digital library from the National Institute of Standards and Technology (NIST, USA) (WILEY.lib), compound identification was automated.

2.4 Structure Retrieval and Preparation

Fatty acid that found tiger milk mushroom extracted from the PubChem Database, and employed as ligands. curcumin, a 3ILS inhibitor served as the standard in this investigation, with its structure retrieved from the PubChem Database [12]. The 3ILS protein structure was obtained from the Protein Data Bank (https://www.rcsb.org/) [10]. Discovery studio software was utilized to eliminate redundant residues, including water molecules and intrinsic ligands, from the retrieved structure.

2.5 Molecular Docking and Visualization

The chosen ligands were subjected to molecular docking studies targeting 3ILS using the Autodock Vina wizard [13] that is included into PyRx [14]. In order to assess and compare the similarity of interactions between the ligands and the standard, a standard molecule was also docked against 3ILS. The molecular docking results were displayed as binding energy scores (in kcal/mol), with a higher affinity denoted by a larger negative number. With coordinates of X:52, Y:58, and Z:44 and dimensions of X:2.667, Y:-0.333, and Z:-0.028, the docking grid encircled the whole protein [15]. With the aid of BIOVIA Discovery Studio, the interactions between the ligands and the amino acid residues of 3ILS were visualized in both 2d and 3d dimensions.

3. Result and Discussion

3.1 Gas Chromatography Results

The GC-MS examination of the ethanol extract from tiger milk mushroom revealed the presence of 30 peaks (Figure 2), with 7 of them identified as fatty acids (Table 1).

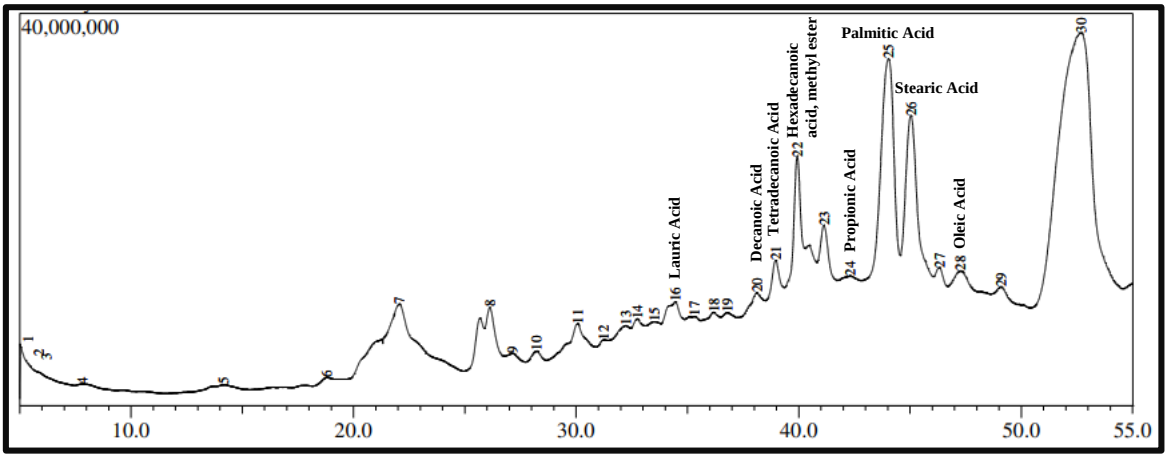


Figure 2. The Chromatogram of the Ethanol Extract from Tiger Milk Mushroom.

Table 1. Fatty Acid Compound from Tiger Milk Mushroom Ethanol Extract

Compound Name	Molecular Formula	Molecular Weight (g/mol)	RT (min)	Area sum %
Lauric Acid	$C_{12}H_{24}O_2$	200	34.469	2.28
Decanoic Acid	$C_{10}H_{20}O_2$	172	38.131	2.53
Tetradecanoic Acid	$C_{14}H_{28}O_2$	228	38.985	2.74
Hexadecanoic Acid-Methyl Ester	$C_{16}H_{32}O_2$	256	39.956	7.97
Propionic Acid	$C_7H_{12}O$	112	42.322	3.10
Palmitic Acid	$C_{16}H_{33}O_2$	256	44.050	13.17
Stearic Acid	$C_{18}H_{36}O_2$	284	45.059	9.24
Oleic Acid	$C_{18}H_{34}O_2$	282	47.277	3.45

The most abundant compound found in the analysis was palmitic acid, representing 13.17% of the total peak area. The seven fatty acids serve various functions. Lauric acid functions as an antibacterial [12], immunomodulator [16], anti-inflammatory [17], antiviral [18], and antifungal [19]. Decanoic acid serves as an energy source [20], antimicrobial [21], and pharmaceutical application [22]. Tetradecanoic acid functions as an insecticide [23], antibacterial [24], and anti-inflammatory [25]. Hexadecanoic acid-methyl ester functions as an antibacterial [26]. Propionic acid functions as an antimicrobial [27], immunomodulator [28], and antifungal [29]. Palmitic acid functions as an energy source [30], cell structure component [31], and hormone production [32]. Stearic acid functions as an emulsifying agent [33], and lubricant [34]. Oleic acid functions as an energy source [35], anti-inflammatory [18], nutrient absorption [36], and skin care [37].

3.2 Molecular Docking

The structural configuration of the ligands required for interaction with the 3ILS protein as well as the strength of their binding, indicated by their binding energy, were predicted using molecular docking analysis (Table 2). Since the suppression of biological activity results from the ligand's attachment to the protein target, binding energy is essential to the inhibitory effect [38]. The binding energy can be used to evaluate the interaction between ligands and the 3ILS protein. Because they require less energy to bind to the protein's binding site, ligands with lower binding energies are thought to be more stable.

Table 2. Molecular Docking Results

Compound	**Binding Energy (Kcal/mol)**
Curcumin	-6,2
Lauric Acid	-3,9
Decanoic Acid	-6,0
Tetradecanoic Acid	-4,9
Hexadecanoic Acid-Methyl Ester	-4,2
Propionic Acid	-3,4
Palmitic Acid	-4,5
Oleic Acid	-4,5

The binding energy of the ligands with 3ILS indicates that all amino acids have binding energies lower than the standard (-6.2 kcal/mol). In this regard, it suggests that they can form stronger and more efficient complexes with 3ILS The ligand with the highest binding energy score is decanoic acid, which results closest to the standard at -6.0 kcal/mol. The presence of unfavorable bonds in ligands with low binding energy suggests that the repulsion effect may be counteracted by other types of interactions, given that the attractive forces from favorable bonds are significantly stronger, resulting in low binding energy [39]. Nevertheless, these unfavorable bonds could still affect the stability of the complex, so their interpretation should be considered alongside the results of molecular dynamics.

3.2 2D Chemical Interactions

The illustration in (Figure 3) show the interaction between residues in decanoic acid and curcumin in 2 d format.

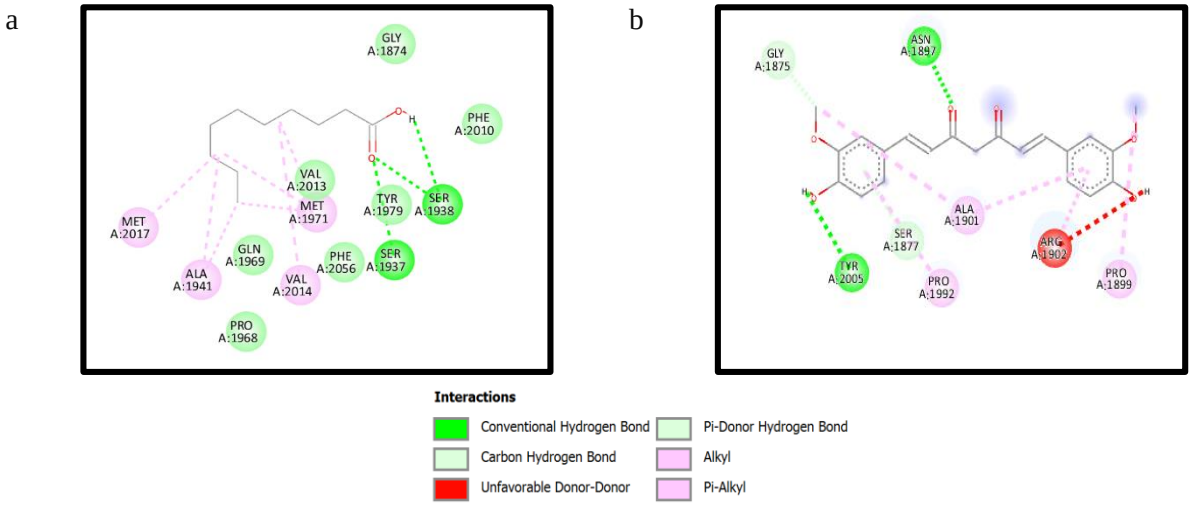


Figure 3. The 3ILS protein is shown in complex with two inhibitors: decanoic acid (a). a competitive inhibitor, and curcumin (b). a selective inhibitor. The dashed lines indicate the established hydrogen bonds with the residues.

Decanoic acid's bonds show both alkyl and hydrogen bonds. SER A: 1938 and SER A: 1937 are the primary amino acids that interact with decanoic acid by traditional hydrogen bonding. The 3ILS domain's active site contained the aforementioned amino acids. Strong chemical interactions with certain amino acids, such as typical hydrogen bonds, consequently result in the suppression of enzyme function [40]. According to reports, decanoic acid might contribute to the fermentate of *A. jensenii*'s antifungal effects [41]. Decanoic acid serves as an energy source [21], has antibacterial qualities [22], and is used in the pharmaceutical sector [23]. Of the substances indicated in (Table 1), decanoic acid has the strongest inhibitory efficacy against fungal protein polyketide synthase.

3.3 Hydrophobic Interactions

Since the interfaces between target and inhibitor complexes widen and cause major changes within the domain, the average amount of hydrophobic atoms emphasizes the importance of hydrophobic interactions in drug design [42]. Intermolecular interactions play a critical role in stabilizing connections between ligands and protein targets, especially in hydrophobic portions of an open conformational environment. For this region to remain flexible, water molecules must be present in these hydrophobic zones [43]. (Figure 4) shows how different inhibitors differ in the hydrophobic and hydrophilic regions of the 3ILS domain.

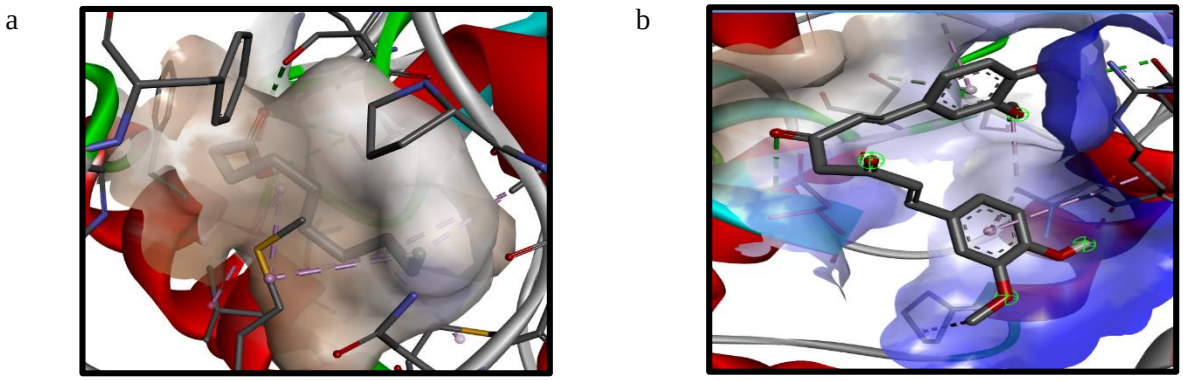


Figure 4. (a).The 3d interaction between decanoic acid and the 3ILS, (b).The 3d interaction between curcumin and the 3ILS.

The brown color mean a significant enlargement of hydrophobic regions, while the blue color indicates the enlargement of hydrophilic regions. Increasing the quantity of hydrophobic particles within the active site of the domain-ligand complex is expected to enhance the ligand's effectiveness in inhibiting biological activity

[44]. The decanoic acid-3ILS complex demonstrated the largest hydrophobic region, represented by the deepest brown shade.

4. Conclusion

Fatty acid compounds (lauric acid, decanoic acid, tetradecanoic acid-methyl ester, hexadecanoic acid, propionic acid, palmitic acid, and oleic acid) isolated from the ethanol extract of *Lignosus rhinocerus* exhibited binding properties with specific residues in the 3ILS domain of *Aspergillus parasiticus*, ranging from -3.4 to -6.0 kcal/mol. Decanoic acid, with most similar binding sites to curcumin, formed two conventional hydrogen bonds and engaged in hydrophobic interactions. This suggests its potential as an inhibitory agent against 3ILS.

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6. Conflict of Interest

The listed writers attest that they have no connections to, or involvement with, any organization or entity that has a financial interest in the topics or materials covered in this manuscript, or any non-financial interest in the topics or materials covered in this manuscript, including connections, knowledge, beliefs, or personal or professional relationships. Honoraria, educational funding, participation in speaker bureaus, memberships, jobs, consulting positions, stock ownership, and other equity interests are examples of financial interests.

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