MOLECULAR DOCKING OF CYMBOPOGON CITRATUS EXTRACTS AS POTENCY FOR DRUG FORMULATION TARGETING FUNGAL SPECIES CAUSING ONYCHOMYCOSIS IN BENUE STATE, NIGERIA

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Abstract

Onychomycosis is a fungal infection of the nails, characterized by thickening, discoloration, and brittleness. It is caused by dermatophytes, non-dermatophyte molds, and yeasts and poses a significant public health challenge, particularly in tropical regions like Nigeria. Current antifungal treatments often show inconsistent efficacy, prompting the search for alternative therapies. This study explores the potential of Cymbopogon citratus (lemongrass) extracts as antifungal agents targeting key enzymes involved in fungal pathogenesis in onychomycosis in Benue State, Nigeria. Using ethical approval and rigorous laboratory protocols, fungal samples were cultured, and the Squalene Epoxidase (SQLE) gene was identified. Phytoconstituents in C. citratus extracts were analyzed via Gas Chromatography-Mass Spectrometry. Molecular docking using the Schrodinger Glide protocol predicted interactions with SQLE, a critical enzyme for fungal ergosterol biosynthesis. Dodecanoic acid-1,2,3-propane showed the highest docking score (-65.13 kcal/mol) and a molecular weight of 638 g/mol, outperforming terbinafine (-64.39 kcal/mol, 327.9 g/mol). The compounds exhibited strong binding to SQLE, suggesting a mechanism for disrupting fungal membrane synthesis. These findings emphasize the integration of computational predictions with in vitro studies in developing effective treatments for onychomycosis, addressing a significant medical need locally and globally.

Keywords: *Cymbopogon Citratus, Molecular Docking, Nigeria, Onychomycosis, Protein–Ligand Interaction, Squalene Epoxidase*

1. INTRODUCTION

Fungal nail infection, known as onychomycosis, is a major issue for public health worldwide, impacting millions of people, especially in countries with tropical climates like Nigeria (Barcellos et al., 2019). The prevalence of onychomycosis is notably high in Benue State, Nigeria, imposing considerable burdens on affected individuals and healthcare systems. Despite various treatment options available, including systemic antifungal agents, topical medications, and physical interventions, the efficacy remains variable, and concerns regarding adverse effects and resistance persist. Hence, there is an urgent need for novel, effective, and safe therapeutic agents to combat onychomycosis.

For a while now, it has been acknowledged that medicinal plants are rich sources of bioactive compounds that have various pharmacological effects, such as fighting off fungal infections (Biswal et al., 2019; Chen et al., 2018). *Cymbopogon citratus*, commonly known as lemongrass and called Toho Gwaza in Tiv, is a tropical plant widely used in traditional medicine for its antimicrobial properties. Its extracts have shown promising antifungal activity against a range of fungal species. However, the precise mechanisms underlying these effects remain unclear.

Molecular docking, a computer-based method, is now being recognized as an important asset in the field of pharmaceutical research and innovation (Dorfmueller et al., 2014). Prediction of the protein-ligand complex binding mode and energy using molecular docking is a valuable tool in computer-assisted drug design. This method has become a significant aspect of CADD. This effective tool can replicate how small molecules and target proteins interact on a very tiny scale, molecular docking can predict how well they bind together and uncover potential ways they work (Edwin et al., 2012). In the context of onychomycosis treatment, molecular docking studies can aid in identifying natural compounds from *Cymbopogon citratus* extracts that possess high affinity and specificity for key fungal targets involved in the pathogenesis of onychomycosis (Gadea et al., 2017).

The goal of this study is to investigate the capability of *Cymbopogon citratus* extracts in providing bioactive elements that can be used in creating innovative antifungal medications which are specifically designed to combat the fungi responsible for onychomycosis in Benue State, Nigeria. Through a comprehensive laboratory analysis and Gas Chromatography Mass Spectrometry (GC-MS) on the antifungal properties of *Cymbopogon citratus* and molecular docking studies, it further seeks to provide insights into the molecular interactions between bioactive constituents of *Cymbopogon citratus* and selected fungal targets implicated in onychomycosis. The effectiveness of drugs in clinical settings relies on the ability of their bioactive ingredients to attach to specific targets and produce pharmacological effects (Gupta & Simpson, 2019). The core of the drug molecules attaching to the amino acid residue of the receptor is to create a drug-receptor complex that engages in interactions, primarily through inter-molecular electrostatic interaction and the formation of ionic bonds, hydrogen bonds, and Van der Waals forces, ultimately leading to the activation or inhibition of the biological functions of the receptors (Hanaa et al., 2012). Exploring innovative drug research can have a major impact on both society and the economy. In this study, we examined the traditional methods of pharmacology and experimental models to evaluate various compounds found in *Cymbopogon citratus* in comparison to terbinafine. Identifying specific targets is crucial in the initial stages of modern drug research and development (Hanif et al., 2010; Huttlin et al., 2017), given that many crucial biological functions in living beings, such as controlling cell growth, building molecules, sending signals, and passing along genetic material, heavily hinge on how proteins and ligands interact and recognize each other (Jorgensen, 1991). Molecular docking is a theoretical approach used to examine how proteins and ligands interact and recognize each other. This method involves analyzing the interactions between small molecule ligands and receptor biomacromolecules to predict how they bind and the strength of their affinity. It is essential for structure-based drug design, as it provides insights into the molecular mechanisms behind pharmacological activities, predicts protein-ligand complex structures, and aids in targeted drug screening (Hamed Khater & Khattab, 2020). Computer-aided drug design, known as CADD, is a technique in computational chemistry that involves using computer simulations, calculations, and predictions to create and improve potential drug lead compounds based on their interactions with receptors. This approach has the potential to significantly enhance the effectiveness of drug testing, decrease the guesswork in research, highlight the benefits of affordability and quick turnaround, and is a crucial tool

in the field of drug research and development (Kitchen et al., 2004). The emergence of forward and reverse molecular docking technologies coincided with advancements in structural biology and computer capabilities. These technologies utilize computer simulations to position small molecules within the binding sites of larger protein targets, then utilize various parameters to predict binding affinity and create models of ligand-receptor interactions. This approach enables rapid screening of unknown compounds and accelerates the process of drug design and discovery (Kpoviessi et al., 2014). By integrating computational predictions with experimental validation, this study endeavors to contribute to the development of effective and safe therapeutic interventions for onychomycosis, addressing a critical unmet medical need in Benue State, Nigeria and beyond.

2. RESEARCH METHODS

2.1. Ethical Consideration

Ethical approval was sought and obtained from the ethics committee of the Benue State Ministry of Health and Federal Medical Centre, Makurdi respectively, with an issuance of letters of ethical clearance approval reference numbers FMH/FMC/HREC/108/VOL.1 and MOH/STA/204/VOL.1/242.

The study utilized a selective random sampling method. A total of 384 individuals over the age of 18, displaying visible signs of onychomycosis, were selected for participation. Samples of nails were sanitized, gathered, and analyzed. Fungal cultures were grown on Sabouraud Dextrose Agar (SDA). The laboratory techniques involved examining and describing the appearance of the isolates through microscopy.

Cymbopogon citratus leaves and stems were harvested freshly early in the morning from some selected farms at Iyakyor settlements in Benue State. it was further authenticated in the herbarium unit of Botany Department, Joseph Sarwuan Tarka University Makurdi. Samples were deposited with voucher identification number FUAM/BOT – MH/23/055. The *Cymbopogon citratus* underwent crude and oil extraction. The leaves and stems were left to dry in the shade under lab conditions for 12 days before being crushed with a clean mortar and pestle to create a powdered plant material. This powder was then stored in a sealed container for future use. To extract the oil, fresh lemongrass leaves and stems were subjected to steam distillation using a specialized apparatus similar to the one mentioned in the study of Kpoviessi et al. (2014) and Kumar et al. (2018). All chemical and solvents used were of analytical grade. The solvent used were hexane, ethyl-acetate, Methanol and Dimethyl sulfoxide (DMSO) of different polarity. Soxhlet Extraction of the Powdered Plant was performed to get purified compounds of the plant in hexane following the method by Kushwaha et al. (2016). The ethyl acetate extraction of the crude was carried out using Maceration method by Lachenmeier (2008). Extraction of the Crude in Methanol was performed following procedure by Morgado et al. (2017). Extraction of Essential oil by Steam Distillation using Clevenger-type apparatus setup for collecting these *C. citratus* essential oil. The method by Kushwaha et al. (2016) was employed. Column Chromatography for Fractionation was performed in silica gel to obtain purified fractions of the *C. citratus* compounds on the solvents and on combination of the solvents as described by Kpoviessi

et al. (2014). Gas Chromatography-Mass Spectrometry (GC-MS) analysis identified the compounds in the *Cymbopogon citratus*.

2.2. Protein preparation

The crystal structure of the Squalene Epoxidase (SQLE) gene as identified by PCR and Gel electrophoresis causing Onychomycosis was downloaded from the protein database (PDB ID:6C6P). Squalene epoxidase (SQLE) is an essential enzyme found in both fungi and humans. It plays a critical role in the biosynthesis of sterols, which are vital components of cell membranes. However, there are significant differences in the function and importance of this enzyme between these two organisms.

2.2.1. In Fungi

In fungal species, squalene epoxidase is a key enzyme involved in the synthesis of ergosterol, which is the principal sterol component of fungal cell membranes. Ergosterol is crucial for maintaining the integrity, fluidity, and functionality of the fungal cell membrane. Inhibition of SQLE in fungi leads to a depletion of ergosterol and an accumulation of toxic squalene, resulting in impaired cell membrane structure and function, ultimately causing cell death. This makes SQLE an attractive target for antifungal drugs.

2.2.2. In Humans

In humans, squalene epoxidase is involved in the biosynthesis of cholesterol, an essential sterol that plays a pivotal role in maintaining cell membrane structure, synthesizing steroid hormones, and producing bile acids. While the fundamental enzymatic activity is similar to that in fungi, the human SQLE has structural and regulatory differences that distinguish it from its fungal counterpart.

2.2.3. Clinical Implications

The presence of SQLE in both fungi and humans has significant implications for drug development and therapy. Antifungal agents such as terbinafine specifically target fungal squalene epoxidase to treat infections like onychomycosis. Terbinafine binds to the fungal SQLE with high affinity, inhibiting ergosterol synthesis without significantly affecting human cholesterol synthesis due to the structural differences between the fungal and human enzymes. This selective inhibition is crucial for the effectiveness and safety of antifungal treatments.

The SQLE gene encoding squalene epoxidase exists in both fungi and humans, serving analogous but distinct roles in sterol biosynthesis. The differences between the fungal and human enzymes allow for the development of selective antifungal therapies that effectively target fungal infections while minimizing adverse effects on human cells.

The Squalene Epoxidase (SQLE) gene was prepared using the Schrodinger Protein Preparation Wizard 19. All missing loops and side chains were filled using Prime, and water molecules beyond 5.00 angstroms (Å) were deleted, heterogeneous states (het states) were generated at a pH of 7.0 ± 2.0. Disulfide bonds and missing hydrogen were added. The protein crystal structure was finally optimized and was subjected to restrained minimization converging atoms to Root Mean Square Deviation (RMSD) of 0.30Å; used

to compare the similarity between two structures, such as a protein's original structure and its conformation after simulation or docking. It measures the average deviation of the atoms' positions.

2.3. Ligand preparation

The arrangement of molecules in 3D was obtained from the PubChem database (Compound CID:9917765) and processed with the Schrodinger Lig-prep-module. Various forms were created based on a pH range of 7.0 ± 2.0, with adjustments made for desalination, tautomerization, and stereoisomer generation; tautomers are different structural forms of a compound that can interconvert by the movement of a proton and a double bond. The preparation process ensures that all relevant tautomers are considered. Stereoisomers are molecules with the same molecular formula and sequence of bonded atoms but differ in the 3D orientation of their atoms.

2.4. Molecular docking

Using Schrodinger Glide docking protocol (Standard Precision); A level of accuracy in Glide docking, balancing computational efficiency with prediction accuracy. The compound's ligand was placed in the proteins' active regions where it is meant to bind. By creating a receptor grid, the coordinates for the active site were established with a VanderWaals radius scaling factor of 1.0 and a partial charge cut off of 0.25. The ligands were sampled flexibly with nitrogen inversions and conformation also sampled, 5000 poses per ligand were generated at the initial phase of docking with the top 400 poses (based on fit and interactions) selected for further refinement through energy minimization, Epik state penalties were added to the docking score. After all the docking and scoring, only the best pose for each ligand (the one with the highest energy and best fit) is retained and reported.

3. RESULTS AND DISCUSSION

3.1. Results

Nine highest peaked compounds from *Cymbopogon citratus* extracts in Methanol, Ethyl-acetate, Hexane and Essential Oil were assessed using Gas Chromatography and Spectroscopy (GC-MS) viz: Benzene- propyl-, Dotriacontane, Oleic acid, Dodecanoic acid-1,2,3-propane, Hexacosonal, Cyclooctasiloxane-hexadecame, Cyclononasiloxane-octadecame, 2-Cyclohexane-1-carboxyaldehy and Dodecane as shown in Table 1.

The docking score (kcal/mol) indicates the binding affinity of each compound towards the target protein. A higher docking score generally suggests stronger binding between the compound and the target protein. The Molecular Mechanics/Generalized Born Surface Area (MMGBSA) dG Bind (kcal/mol) represents the calculated binding free energy of each compound after docking; similarly, higher values indicate stronger binding.

Dodecanoic acid-1,2,3-propane had the highest docking score (-9.71 kcal/mol) and a relatively high MMGBSA dG Bind value of -65.13 kcal/mol. with Molecular weight = 638g/mol. indicating strong binding to the target protein. Terbinafine, used as a control

compound, had a docking score of -6.222 kcal/mol and an MMGBSA dG Bind of -64.39 kcal/mol with molecular weight = 327.9 g/mol. suggesting moderate binding affinity.

Dotriacontane displayed a docking score of -4.737 kcal/mol and an MMGBSA dG Bind of -49.17 kcal/mol, indicating weaker binding compared to Terbinafine. Other compounds, such as Hexacosonal, Oleic acid, Benzene-propyl-, Cyclooctasiloxane-hexadecame, Cyclononasiloxane-octadecame, 2-Cyclohexane-1-carboxaldehyde, and Dodecane, show various docking scores and binding energies, suggesting differing degrees of interaction with the target protein.

Overall, Dodecanoic acid-1,2,3-propane appears to be the most promising compound based on these docking results, as it exhibits the strongest binding affinity among those tested, as presented in Table 2.

Table 1. GC-MS Analysis of *Cymbopogon citratus* in Methanol, Ethyl-acetate, Hexane and Essential Oil

RT (s)	Area Peak (%)	% Average Height	Compound
3.522	0.09	0.99	Benzene, propyl-
24.59	60.56	2.31	Dotriacontane
19.42	21.80	4.14	Oleic Acid
17.54	31.79	6.48	Dodecanoic acid, 1,2,3-propane
27.73	30.98	2.58	Hexacosonal
12.59	70.51	3.48	Cyclooctasiloxane, hexadecame
24.73	50.70	1.94	Cyclononasiloxane, octadecame
6.83	90.32	1.99	2-Cyclohexene-1-carboxaldehy
7.144	0.37	2.30	Dodecane

Table 2. Molecular Docking

Compound name	Docking score(kcal/mol)	MMGBSA dG Bind(kcal/mol)
Dodecanoic acid-1,2,3-propane	-9.71	-65.13
Terbinafine (control)	-6.222	-64.39
Dotriacontane	-4.737	-49.17
Dodecane	-5.702	-46.03
2-Cyclohexene-1-carboxaldehy	-3.201	-44.34
Benzene-propyl-	-4.399	-42.78
Oleic Acid	-3.36	-41.74
Cyclooctasiloxane, hexadecame	-2.791	-42.25
Hexacosonal	-2.92	-48.25
Cyclononasiloxane, octadecame	-3.86	-44.68

In order to conduct more in-depth molecular docking investigations, the method of binding involving interactions similar to a lock and key mechanism, as well as affinity measured by Vina scores, was established using AutoDock/Vina. The results of the docking process indicated that the binding scores for all substances exceeded -2.791 kcal/mol. Among these results, the most favorable interaction was observed between

Dodecanoic acid-1,2,3-propane and the derivative Hydrogen (H-bonds) with PHE 166 and Glu168 in the Squalene epixodase (SQLE) gene.

Abbreviations of amino acids: Alanine (ALA), Cysteine (CYS), Glycine (GLY), Lysine (LYS), Leucine (LEU), Arginine (ARG), Serine (SER), Threonine (THR), Tyrosine (TYR), Phenylanine (PHE).

The best interaction with enzymes was generated with Hydrogen-bonds forming at PHE 166, GLN 168, LEU 333, TRY 335, THR 417, LEU 473, VAL 506, PRO 415, LUE 416, LEU 509, GLY 418, LEU 334, LEU 345, PHE 477 and VAL 506 whereas the Terbinafine produced LEU 324, PHE 477, LEU 476, LEU 473, TYR 195, LEU 197, PHE 495, TRY 494, CYS 491, VAL 526, PHE 523, HIS 522, LEU 469, LEU 519 respectively and Dodecane in the active site of SQLE were PHE 166, GLN 168, PRO 415, LEU 416, THR 417, GLY 418, LEU 473, VAL 502, PRO 505, VAL 506, LEU 509, TYR 195, ILE 197, LEU 324, ALA 322 and GLU 323 also 2D docking pose of Dotriacontane in the active site of SQLE resulted in ALA 322, GLY 418, THR 417, LEU 416, PHE 477, CYS 491, VAL 526, PHE 523, HIS 522, LEU 519, VAL 518, PRO 505, VAL 506, LEU 508, LEU 509, GLN 168 and PHE 166 as presented in figures 1,2,3 and 4 respectively. Therefore, results suggests that *Cymbopogon citratus* compounds be considered as an important lead for formulating drugs that can be used for the treatment of onychomycosis.

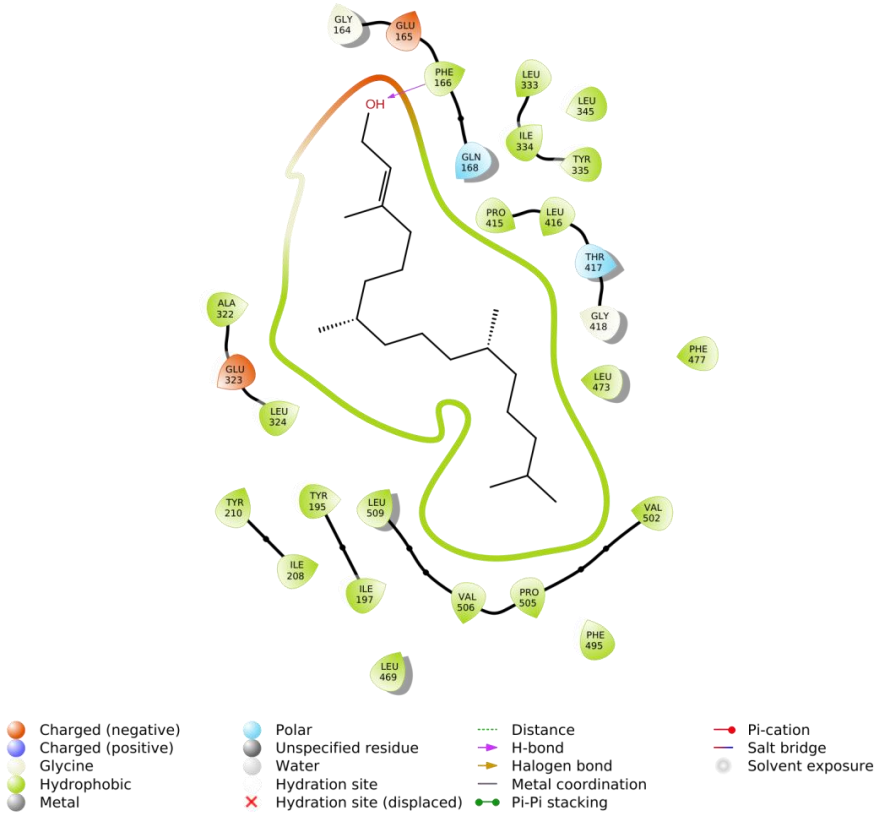

Figure 1. 2Ddocking pose of Dodecanoic acid-1,2,3-propane (-9.71) in the active site of SQLE: Binding Affinity-65.13kcal/mol

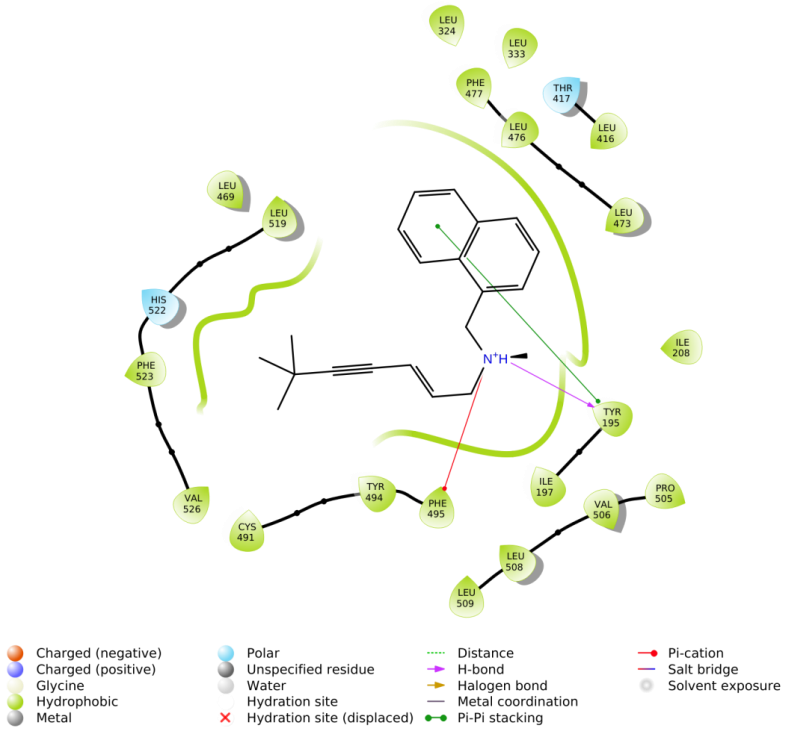

Figure 2. 2D docking pose of Terbinafine (-6.222) in the active site of SQLE: Binding Affinity -64.39kcal/mol

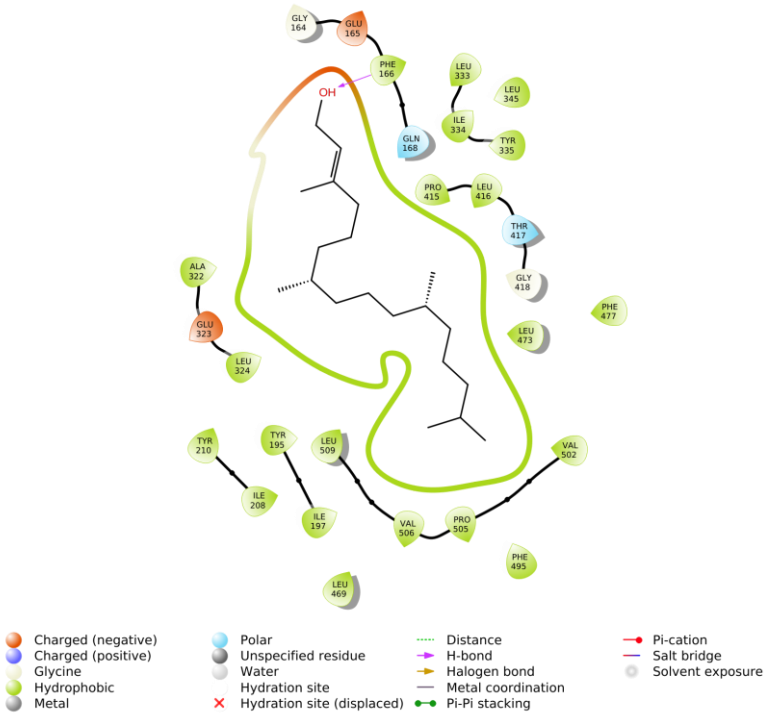

Figure 3. 2Ddocking pose of Dodecane (-5.702) in the active site of SQLE: BindingAffinity-46.03kcal/mol

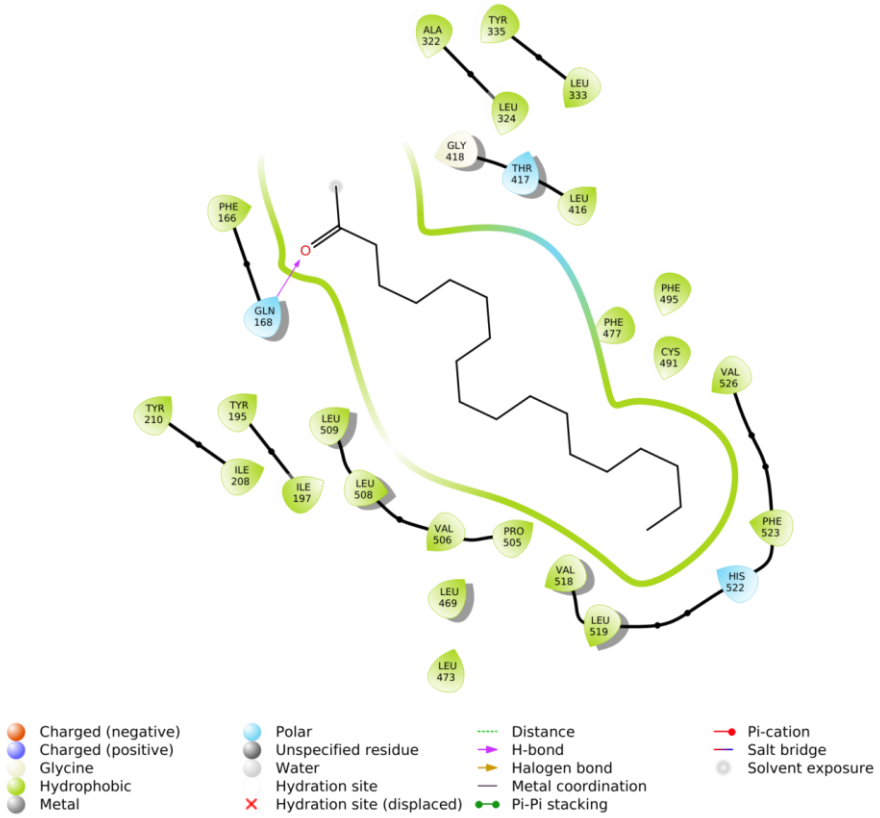

Figure 4. 2Ddocking pose of Dotriacontane (-4.737) in the active site of SQLE: Binding Affinity-49.17kcal/mol.

3.2. Discussion

The plants of *Cymbopogon citratus* generate terpenoidal hydrocarbons and essential oils that can be categorized for medicinal, industrial, or perfumery uses based on their chemical makeup. Among these compounds, dodecanoic acid and 1,2,3-propane showed the highest percentage average height at 6.48% and an area peak at 13.79% at a retention time of 17.543s. In contrast, benzene and propyl- exhibited the lowest percentage average height at 0.99% and an area peak of 0.09% at 3.522s, respectively. The outcome mirrors the discoveries of Edwin et al. (2012) who found that nine components were separated from the GC column of *Cymbopogon citratus* essential oil, each with varying retention times. Previous studies have also identified some of the compounds detected in this study as being present in *Cymbopogon citratus* extracts. Ali et al. (2017) reported the presence of citral (34.8%), neral (30.72%), β-myrcene (11.28%), geraniol (5.54%)1,3,4-trimethyl -3- cyclohexene-1-carboxaldehyde (2.20%), and citronellol (1.34%). Geranyl acetate (0.57%), heptane-2- Carboxaldehyde-6,6-dimethyl (0.23%) and D-lemonene as major components of essential oils of *C. citratus* extract using GC-MS. The findings of this study also agree with the report of Maqbul et al. (2022) who in their study confirmed the presence of Octadecanoic acid, Hexadecanoic acid, 2-Cyclohexane-1- carboxaldehyde among other compounds to be present in the essential oil of *C. citratus* extract using GC-MS.

Similarly, in a more recent study, it has been reported that the water distillation extract of lemongrass stems contains various types of essential oil chemical compounds, such as citronellal, citronellol and graniol, where based on the results of the analysis that have been analyzed from the GC results, 13 peaks were detected as chemical compounds of essential oil groups after being analyzed into the form of essential oil grouping based on GC and MS information, 3 peaks of chemical compounds were obtained which had the highest percentage area where the essential oil compounds 2,6-Octadional acid, 3,7-dimethyl-, (z)- citronellal group had the highest peak percentage area of 14. 83% retention time 7.86 min (Mulabagal & Calderón, 2010).

Docking mode revealed that the –OH groups of these compounds acted as the source of hydrogen bonds for the protease residues. The hydrogen bonds between these compounds and the side chains of TYR 210 and ILE 208 in the SQLE gene, as well as the hydrophobic interactions with the residues, played a role in stabilizing the ligand–receptor complexes. A higher docking score generally suggests stronger binding between the compound and the target protein. MMGBSA dG Bind (kcal/mol) represents the calculated binding free energy of each compound after docking. Similarly, higher values indicate stronger binding.

Structure-based drug design (SBDD) is the most frequently employed method, being centered on the three-dimensional structure (Pereira & Aires-de-Sousa, 2018). Molecular docking is an important method used in SBDD for creating new drugs. In-silico docking allows scientists to analyze the shapes and strengths of various bioactive compounds when interacting with receptors (Sharma & Kaur, 2022).

In a similar study conducted by Sharma & Kaur (2022), it was reported that the citral bioactive molecule found in lemon grass oil was identified as a potential fungal inhibitor for key enzymes such as chitin synthase, UDP-glycosyltransferase, and Glucosamine-6-phosphate synthase. Research has shown that citral effectively bonded with the active sites of these enzymes. Chitin synthase is an enzyme complex located in the membrane, consisting of three sections: a starting domain, a functional domain, and a terminal transmembrane domain. Through computer analysis, it was discovered that citral interacts with the active domain responsible for extending chitin chains between the starting and ending domains (Singh et al., 2016). The molecular docking results indicate that Dotriacontane, Dodecane, terbinafine, and Benzene propyl possessed superior binding energy with a-glucosidase (binding affinity: -46.03kcal/mol, -49.17kcal/mol, -64.39kcal/mol, and -65.13kcal/mol respectively). This is similar to the findings of Wang et al. (2022), who in their study reported that 1-O-p-coumaroyl-3-O-caffeoylglycerol (8) and 1,3-O-dicaffeoylglycerol (9) possessed superior binding energy with a-glucosidase (binding energy: -5.19, -5.97 kcal/mol, respectively) The molecular docking results showed that 1-O-p-coumaroyl-3-O-caffeoylglycerol (8) and 1,3-O-dicaffeoylglycerol (9) possessed superior binding energy with a-glucosidase (binding energy: -5.19, -5.97 kcal/mol. When the drug molecule docks, it can either make hydrophobic connections or hydrogen bonds with the active site residues of the receptor, which in turn influences the strength of the bond between the ligand and receptor. Nine most active compounds were identified from leaves and stems of *C. citratus* in this study. This result does not correspond to previous study that reported twenty most active compounds isolated and identified from the dried aerial part of *C. citratus* (Suresh et al., 2008). This could be

attributed to variation in the species of the plant, part of the plant, seasonal/regional variations, methods of extraction among several other factors. However, the finding of this study agrees with Omar et al. (2021) and Biswal et al. (2019) who stated in their study that the antifungal properties of key compounds found in essential oils from Trachyaspermum ammi, Thymus vulgaris, and Boswellia carteri were investigated through molecular docking analysis. The findings suggest that *Cymbopogon citratus* extracts, particularly its essential oil, could be a significant source of antifungal agents.

4. CONCLUSION

The results strongly demonstrate the great potential of Dodecanoic acid-1,2,3-propane**,** Benzene-propyl, Dodecane and Dotriacontane compounds for future clinical usage against onychomycosis and also take the forefront in exploring and creating pharmaceuticals derived from fungi. Finally, the energy scores of the tethered *Cymbopogon citratus* compounds were found to be superior to those of the terbinafine (control) typically prescribed for onychomycosis treatment.

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