How to Cite:
Sriwastava, A. R., & Srivastava, V. (2022). Identification of bioactive agent in tinospora cordifolia by in- silico approach. *International Journal of Health Sciences*, *6*(S3), 5968–5979. https://doi.org/10.53730/ijhs.v6nS3.7313

Identification of bioactive agent in tinospora cordifolia by in- silico approach

Akanksha Raj Sriwastava
Department of biotechnology, Faculty of Engineering and Technology, Rama University, G. T. Road, Kanpur-209217-India

Vivek Srivastava
Department of biotechnology, Faculty of Engineering and Technology, Rama University, G. T. Road, Kanpur-209217-India
Corresponding author email: viveksrivastavabio@gmail.com

Abstract*---Candida*** species cause most fungal infections and contribute significantly to worldwide morbidity and mortality, making them a severe public health threat. Due to their ability to develop resistance to antifungal drugs, these opportunistic fungi defy therapeutic efforts, making them a severe problem in treating and managing Candida infections. Due to co-infection with immune-compromised persons, multidrug-resistant Candida spp. strains have developed as a global concern, which can lead to invasive candidiasis. The life-threatening variant of the illness may be treated quickly and effectively through drug repurposing. Hence, this research was done in tandem with a previous inquiry into the chemicals' ability to fight Candida spp. According to molecular docking and molecular dynamics studies, a total of five compounds, namely Cholesterol, Allopyranose, Melezitose, 1,6-Anhydro-B-D-Glucofuranose, and 1-(3-Cyanophenly)-2-Phenylethane isolated from the sample (in previous study) have the potential to inhibit the growth of further Candida albicans. After ligand binding, the protein-ligand interaction was also studied to know which residues are involved in bond formation. Out of these five ligands, only one ligand violated Lipinski's rule, namely Melezitose. Therefore, the compounds isolated in the previous study have a strong antifungal effect. However, molecular dynamics simulation and in-vitro investigation are still needed to back up these claims.

***Keywords*---**Candida, molecular docking, ligand, antifingal interactions.

Introduction

Candida is a widespread genus that may infect up to 70% of the population and cause no symptoms. Local or systemic alterations, on the other hand, may induce host instability and predispose individuals to candidiasis, which can range from a minor infection to a catastrophic illness (Singh *et al.*, 2014; Singh *et al.*, 2022). Candida infections are frequent in the oral cavity, especially in immunocompromised individuals, those who wear dental prosthetics, and those who have hyposalivation (Billings *et al.*, 2017). Candida krusei, Candida tropicalis, Candida glabrata, and Candida albicans are the bacteria that cause oral candidiasis. Biofilms can be formed in the oral mucosa, dental tissues, restorative materials, and denture prosthesis by these species (Billings *et al.*, 2017; Muadcheingka and Tantivitayakul, 2015).

The most often used antifungal classes in conventional therapy are polyenes (nystatin and amphotericin B) and azoles (miconazole, clotrimazole, ketoconazole, itraconazole, and fluconazole). Voriconazole and posaconazole (triazoles), liposomal amphotericin B (liposome integrated), or echinocandin may be necessary in some cases (Patil *et al.*, 2015; Yusufkhan *et al.*, 2022). Nystatin (as an oral solution) and miconazole are the most often used topical therapies for superficial infections. If topical medicine fails, systemic ketoconazole and fluconazole treatment is advised (Manik and Bahl, 2017). The primary issue is that resistance to these drugs has evolved due to indiscriminate preventative use and self-medication (Junqueira *et al.*, 2012). To overcome microbial resistance and drug toxicity (in cellular, tissue, neurological, renal, or hepatic), natural compounds with therapeutic qualities have been extensively studied as a potential alternative to traditional antifungal medication (Fox *et al.*, 2013). Naturally occurring chemicals with great effectiveness against pathogenic fungus can be identified and chemically created (Doddanna *et al.*, 2013). Natural commodities, such as essential oils and their components, have been associated with cheaper pricing, less toxicity, and more affordability when compared to prescription drugs. However, due to fungal resistance and a failure to extract the active principle(s), other treatments may be less effective in terms of antibacterial activity (Barbosa *et al.*, 2014). Long-term therapy may be necessary in this scenario to achieve full recovery. Antifungal resistance of C. albicans has been associated to long-term use of antifungal medications and recurrent mucocutaneous and oropharyngeal candidiasis. Candida species like C. krusei are naturally resistant to a variety of antifungal medicines, including fluconazole. When new types of antifungal medications were developed, resistance spread faster, especially when azoles (such as fluconazole) were utilised (Arendrup *et al.*, 2017).

In-silico pharmacotherapy and computer-aided drug design (CADD) are rapidly growing topics that include the creation of software tools for acquiring, analysing, and combining biological and medical data from many sources. Pharmaceutical sciences and other disciplines of study have made therapeutic agent screening considerably more accessible, with high-throughput findings in a short amount of time (Joseph *et al.*, 2017). Furthermore, bioinformatics technologies enable a better understanding of the biological effect by providing a deeper knowledge of the target-receptor relationship (Xiao *et al.*, 2015). Identifying possible therapeutic

compounds entails a number of steps, beginning with the selection of a disease, followed by the identification of a suitable target molecule, the creation of a small molecule library, and target ligand interaction studies. Molecular docking is the most often used approach for predicting the network linking the protein and the ligand, as well as the modalities by which the ligand binds and inhibits the protein, in drug design based on structure (Sethi *et al.*, 2019; Dibha *et al.*, 2022). Although molecular docking is a rapid approach for determining the binding mode of any ligand at the active site of a protein, the results are not without flaws. As a result, simulation is followed by docking, since simulations in the system are simulated when thermal fluctuations are present. As a result, one protein from Candida albicans was chosen for this investigation based on its relevance, and 30 plant-based compounds (obtained from a previous study using GC-MS) were chosen to find the most appropriate and important lead molecule with the best binding energy and maximum binding affinity. This research also combines ADMET analysis, molecular docking, and protein-ligand interaction analysis.

Materials and Methods

Protein and ligand retrieval

The selection of the protein and ligand plays an integral role in the entire process of CADD as they act to carry out all the biological functions in our body. This study selected one protein from *Candida albicans* based on protein's importance. The acetohydroxyacid synthase (AHAS) enzyme of *Candida albicans* is the first enzyme in the branched-chain amino acid biosynthesis pathway. Hence, considering the importance of the above-stated protein, the selected target proteins of the study were browsed in the structural database RCSB-PDB (Berman *et al.*, 2000) to obtain their 3D structure. *Candida albicans* acetohydroxy acid synthase crystal structure in combination with the herbicide iodomuron methyl had PDB ID: 6DEO were downloaded using RCSB-PDB. A total of thirty small molecules obtained through GC-MS analysis were selected as potent ligand molecules. Structures of ligands chosen were obtained in SDF format from PubChem (https://pubchem.ncbi.nlm.nih.gov), a database for chemical compounds, their structure, properties, and activities.

Protein and Ligand Preparation

Appropriate selection and processing of coordinates for receptors and ligands are essential factors in the successful docking process. These preparation processes require conversion of PDB file format to PDBQT file format. The 3D structure of both the selected protein were then prepared for molecular docking. All water molecules, ions, and ligands were removed from the protein molecule using PyMOL software for preparation. AutoDock software was used to prepare the required files for AutoDock Vina by assigning hydrogen polarities, calculating Gasteiger and Kollman charges to proteins and ligands structures (Lim *et al.***,** 2011; Jaghoori *et al.***,** 2016).

Molecular Docking and Protein-Ligand Interaction

Molecular docking may be defined as an optimization problem, which would describe the "best-fit" orientation of a ligand that binds to a particular protein of interest and is used to predict the structure of the intermolecular complex formed between two or more molecules. There are several possible mutual conformations in which binding may occur. These are commonly called binding modes (Sharma *et al.*, 2010)**.** In modern drug design, molecular docking provides valuable information about drug-receptor interactions. It is frequently used to predict the binding orientation of small molecule drug candidates to their protein targets to predict the small molecule's affinity and activity.

A grid box was generated, keeping the ligand as a center (the active site was given in the literature) to locate the coordinates for the ligand's possible binding location in the receptor's active site pocket. The configuration file "conf.txt" was prepared from the grid output file. Autodock Vina docking tool was used for molecular docking that takes the atomic coordinates of the target protein and selected ligand, thus predicting the most suitable docking conformation of the two. During the entire docking process, the target (protein) was kept rigid while ligands were flexible with the aim to determine the best suitable pose. Autodock vina was run on the command line. The protein and ligand files' directory was accessed, vina_split and vina.exe were stored in the same directory, and the command given below was used to run the autodock vina program. An output log file with all the binding energies between protein and different orientations of ligand was further analyzed. On completion of the Docking process, the highest-ranking poses were selected for further protein-ligand interaction analysis. The interaction was visualized on LigPlot+ v.1.4.5 program and BIOVIA Discovery Studio Visualizer 2020 (Srivastava *et al.*, 2021) and LigPlot+ v.1.4.5 program. These programs help project 3D structure to a 2D image, thus facilitating close inspection of 2D Hydrogen and hydrophobic interactions of the protein-ligand complex.

Drug-likeness property of ligands

DruLiTo open-source software used to calculate drug-likeness property in order to determine the cytotoxicity activity of substances in humans. A ligand's pharmacological importance is determined by its drug bioavailability or drug-likeness, which is based on physiochemical and structural features. DruLiTo software was used to analyse all ligands for their drug-likeness properties using Lipinski's five guidelines (Leeson, 2012). The 30 molecular structures found from the database were combined into a single.sdf file using the OpenBabel file format converter, which is a suitable file format for DruLito. The merged file was then submitted to DruLito's website, and only the Lipinski rule of 5 was used to screen the molecules. The computed properties were then saved to the chosen location as a.csv file. The file-save Lipinski filtered molecule option was used to store the structure of the molecules that passed the filter.

Results and Discussions

Analysis of Molecular Docking

A molecular docking study was performed against *Candida albicans* with PDB ID: 6DEO. Further, Grid center and size for protein having PDB ID: 6DEO are center_x = 56.862, center_y = 68.632, center_z = 64.948, size_x = 40, size_y = 40, and size_z = 40. Table 1 below shows the binding energy of two molecular targets with thirty small molecules obtained through GC-MS analysis in our previous study. The significant step of the CADD protocol includes predicting the affinity of the binding of a ligand (Nguyen *et al.*, 2019), where the binding affinity is defined as the binding equilibrium free energy amid two molecules (Lazaridis, 2002). Five ligands are found to have binding affinity below -6.0 kcal/mol against the desired selected protein. The best binding affinity is shown by ligand cholesterol which is -7.1 kcal/mol against *Candida albicans* having PDB ID: 6DEO. Thus, we can say that five compounds namely Cholesterol, Allopyranose, Melezitose, 1,6-Anhydro-B-D-Glucofuranose, and 1-(3-Cyanophenly)-2-Phenylethane isolated from the *Tinospora cordifolia* have the potential to inhibit the growth of further *Candida albicans. Hence,* protein-ligand interaction analysis was performed with this ligand only in complex with each targeted protein.

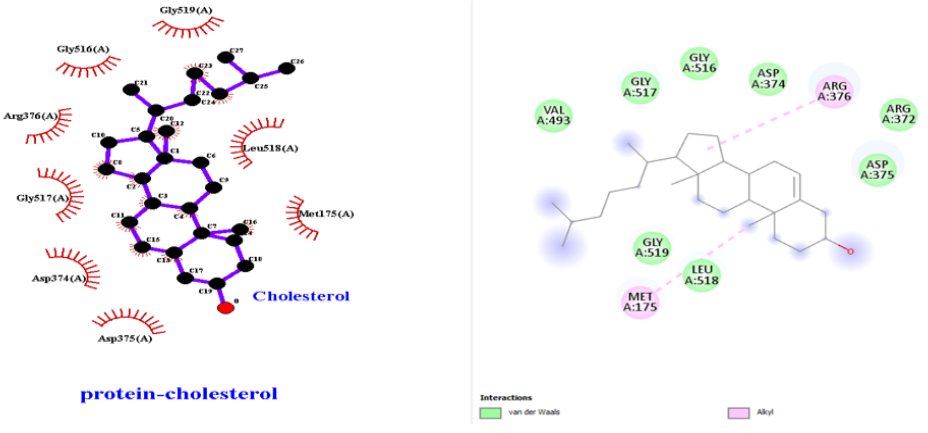
Table 1
Binding energy of thirty ligands against protein of *Candida glabrata protein having* PDB ID: 6DEO

S.No.	PubChem CID	Compound Name	Binding Affinity (kcal/mol)
1.	5997	Cholesterol	-7.1
2.	439507	Allopyranose	-6.4
3.	92817	Melezitose	-6.3
4.	552317	1,6-Anhydro-B-D-Glucofuranose	-6.2
5.	141877	1-(3-Cyanophenyl)-2-Phenylethane	-6.1
6.	6329236	Dimethyl(1-Cyclopentylethoxy)Silane	-6.0
7.	14014	DIMETHYLSILANEDIOL	-5.9
8.	180508	Tris(Trimethylsilyl) Ester	-5.7
9.	985	Palmitic Acid	-5.6
10.	5281	Stearic Acid	-5.4
11.	538757	2,4-dihydroxy-2,5-dimethylfuran-3-one	-5.4
12.	699486	Levoglucosenone	-5.3
13.	445639	Oleic Acid	-5.3
14.	119838	3,5-dihydroxy-6-methyl-2,3-dihydropyran-4-one	-5.3
15.	3840	Kojic Acid	-5.3
16.	11748436	methyl (9Z,11E)-octadeca-9,11-dienoate	-5.2
17.	6161490	Trans-13-Octadecenoic Acid	-5.1
18.	5284421	METHYL LINOLEATE	-5.0
19.	85994440	2-[2-(2-butoxyethoxy)ethoxy]acetic acid	-4.9
20.	237332	5-HYDROXYMETHYLFURFURAL	-4.9
21.	28455	4-METHYLNONANE	-4.7

22.	21205	2-(2-(Diethylamino)Ethylamino)-4-Methylpyridine	-4.7
23.	12097	5-METHYLFURFURAL	-4.6
24.	5364422	8-Octadecenoic Acid	-4.5
25.	519466	1-(furan-2-yl)-2-hydroxyethanone	-4.5
26.	88095	Methoxyacetic Anhydride	-4.5
27.	70259	2,4-Dimethylimidazole	-4.4
28.	76662	1-(furan-2-yl)propan-1-one	-4.3
29.	7362	FURFURAL	-4.0
30.	10341	2(5H)-Furanone	-3.8

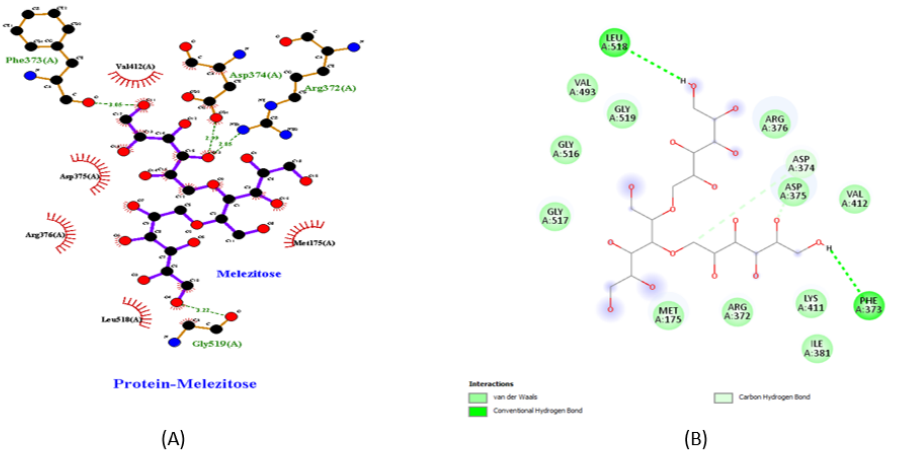
Protein-Ligand Interaction study

Protein with PDB ID 6deo in complex with five best docked ligands were subjected to study interaction analysis using BIOVIA Discovery Studio Visualizer 2020 (Srivastava *et al.*, 2021) and LigPlot+ v.1.4.5 program. Figures 1 show interaction between 6deo and Cholesterol; Figure 2 show interaction between 6deo and Melezitose;; Figure 3 show interaction between 6deo and Allopyranose; Figure 4 show interaction between 6deo and 1,6-Anhydro-B-D-Glucofuranose; Figure 5 show interaction between 6deo and 1-(3-Cyanophenly)-2-Phenylethane; by BIOVIA Discovery Studio Visualizer 2020 and LigPlot+ v.1.4.5 program. Images from BIOVIA Discovery Studio Visualizer 2020 show various colors that depict different interactions- Light green: Vander Waals interaction; Dark green: conventional Hydrogen bonds; Fluorescent green: Piloan Pair; Pink: Pi-alkyl bond; Dark yellow: Pi-cation; Bright yellow: Pi-sulfur and Red: unfavorable accepter-accepter. The interaction code present in the figures obtained from LigPlot+ v.1.4.5 program is depicted in Figure 6.



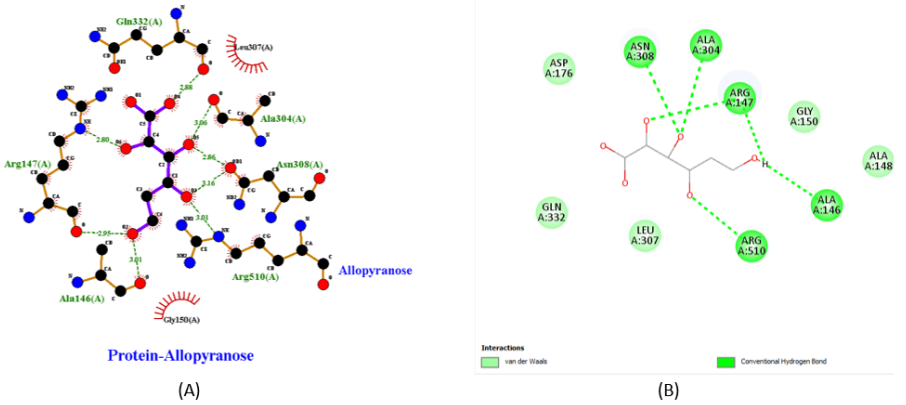
(A) (B)

Figure 1. (A); Interaction between 6DEO and Cholesterol using LigPlot+ v.1.4.5 program; (B): Interaction between 6DEO and Cholesterol using BIOVIA Discovery Studio Visualizer 2020



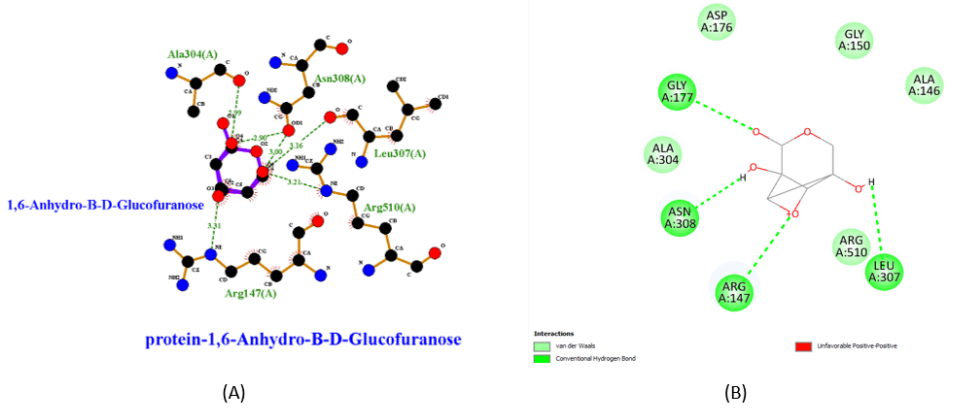
(A) (B)

Figure 2. (A); Interaction between 6DEO and Melezitose using LigPlot+ v.1.4.5 program; (B): Interaction between 6DEO and Melezitose using BIOVIA Discovery Studio Visualizer 2020



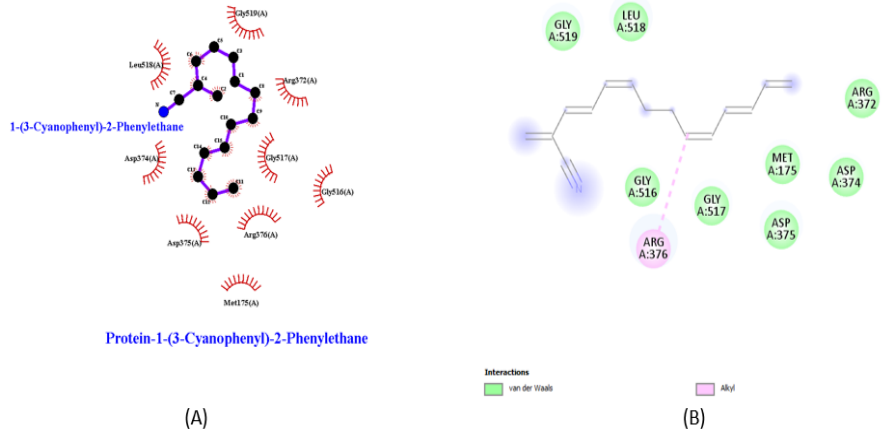
(A) (B)

Figure 3: (A); Interaction between 6DEO and Allopyranose using LigPlot+ v.1.4.5 program; (B): Interaction between 6DEO and Allopyranose using BIOVIA Discovery Studio Visualizer 2020



(A) (B)

Figure 4. (A); Interaction between 6DEO and 1,6-Anhydro-B-D-Glucofuranose using LigPlot+ v.1.4.5 program; (B): Interaction between 6DEO and 1,6-Anhydro-B-D-Glucofuranose using BIOVIA Discovery Studio Visualizer 2020



(A) (B)

Figure 5. (A); Interaction between 6DEO and 1-(3-Cyanophenly)-2-Phenylethane using LigPlot+ v.1.4.5 program; (B): Interaction between 6DEO and 1-(3-Cyanophenly)-2-Phenylethane using BIOVIA Discovery Studio Visualizer 2020

Figure 6. Interaction key for LigPlot+ v.1.4.5 program

Drug-likeness property analysis of ligands

Thirty isolated small molecules were chosen from the PubChem database by considering their property, and then the SDF file of all molecules was downloaded from the same database. The oral route of administering a drug in humans can be determined by Lipinski's rule of five [Lipinski *et al.*, 2001]. The SDF files of these selected 30 molecules were converted to PDB files using Open Babel, then after the screening of molecules using DruLito software was performed, which identified 28 molecules that had suitability for lead molecules owing to follow Lipinski rule of 5. Table 2 below shows all 30 molecules' PubChem CID, including the properties. The light orange rows highlight those molecules that disobey the Lipinski rule in Table 2. From the results it is clear that out of the best five ligands four ligands are following Lipinski's rule of five and only one ligand which is Melezitose violate the Lipinski's rule of five.

Table 2
Physio-chemical properties of selected ligands

Sr. No.	Title	MW	logp	Alogp	HBA	HBD	TPSA	AMR	nRB	nAtom	nAcidicGroup	RC
1	6329236	171.12	0	0	1	0	9.23	0	3	30	0	1
2	5364422	296.27	8.513	-2.092	2	0	26.3	73.69	16	57	0	0
3	180508	342.05	5.004	6	3	0	27.69	56.87	6	43	0	0
4	5281	284.27	8.708	-4.474	2	1	37.3	61.34	16	56	1	0
5	85994440	220.13	0.372	-1.371	5	1	64.99	51.92	11	35	1	0
6	11748436	294.26	8.069	-1.048	2	0	26.3	78.18	15	55	0	0
7	6161490	282.26	8.192	-2.343	2	1	37.3	68.92	15	54	1	0
8	5284421	294.26	8.186	-0.698	2	0	26.3	80.14	15	55	0	0
9	699486	126.03	-0.326	-0.112	3	0	35.53	30.13	0	15	0	2
10	552317	162.05	-1.966	-1.747	5	3	79.15	32.41	0	21	0	2
11	538757	144.04	0.574	-0.909	4	2	66.76	34.71	0	18	0	1
12	519466	126.03	-0.438	-1.112	3	1	46.53	33.3	2	15	0	1
13	445639	282.26	8.192	-2.343	2	1	37.3	68.92	15	54	1	0
14	439507	180.06	-1.697	-2.513	6	5	110.38	35.92	1	24	0	1
15	237332	126.03	-0.383	-0.753	3	1	46.53	34.48	2	15	0	1
16	141877	207.1	3.406	2.379	1	0	23.79	74.18	3	29	0	2
17	119838	144.04	0.327	-1.126	4	2	66.76	34.55	0	18	0	1
18	92817	504.17	-5.243	-6.057	16	11	268.68	101.19	8	66	0	3
19	88095	162.05	-0.345	-0.722	5	0	61.83	34.99	6	21	0	0
20	76662	124.05	0.852	-0.364	2	0	26.3	36.62	2	17	0	1
21	70259	96.07	0.434	-1.082	2	1	24.39	28.78	0	15	0	1
22	28455	142.17	5.969	-0.405	0	0	0	40.88	6	32	0	0
23	21205	270.26	7.83	-2.006	2	0	26.3	64.37	14	53	0	0
24	14014	92.03	0.122	0.245	2	2	40.46	17.02	0	13	0	0
25	12097	110.04	0.654	0.135	2	0	26.3	32.94	1	14	0	1
26	10341	84.02	0.308	0.282	2	0	26.3	21.25	0	10	0	1
27	7362	96.02	0.418	-0.379	2	0	26.3	27.12	1	11	0	1
28	5997	386.35	10.518	1.555	1	1	20.23	115.17	5	74	0	4
29	3840	142.03	-0.13	-1.569	4	2	66.76	34.98	1	16	0	1
30	985	256.24	7.57	-3.898	2	1	37.3	55.52	14	50	1	0

Conclusion

Candida species are the most common yeast infections capable of entering the human digestive system. Until the co-existing microbiota manages them, these pathogens can survive as commensals without creating problems. They manifest themselves as superficial infections in hospitalised or immune-compromised patients shortly after surgery (Bassetti *et al.*, 2018). Oral thrush, vaginal thrush,

genital infections, skin infections, urinary tract infections, and bloodstream infections can all be caused by opportunistic candida spp. growth and dissemination. Thus, this study was carried out with reference to our previous study to find the compounds' ability to work against *Candida albicans*. Cholesterol, Allopyranose, Melezitose, 1,6-Anhydro-B-D-Glucofuranose, and 1-(3-Cyanophenly)-2-Phenylethane, all recovered from the isolated sample in previous study, have the ability to suppress the development of Candida albicans, according to molecular docking and molecular dynamics investigations. Following ligand binding, researchers looked at the protein-ligand interaction to see which residues are involved in bond formation. Only Melezitose, one of the five ligands, broke Lipinski's rule of five. As a result, the chemicals discovered in the previous investigation might have potent antifungal properties. However, a molecular dynamics simulation and in-vitro study are still required to prove these results strongly.

References

1. Arendrup, M. C., & Patterson, T. F. (2017). Multidrug-resistant Candida: epidemiology, molecular mechanisms, and treatment. *The Journal of infectious diseases*, *216*(suppl_3), S445-S451. https://doi.org/10.1093/infdis/jix131
2. BARBOSA, M. B., & FARIA, M. G. I. (2014). Produtos Naturais Como Nova Alternativa Terapêutica Para o Tratamento de Candidíase Bucal. *Uningá Review Journal*, *20*(1). http://34.233.57.254/index.php/uningareviews/article/view/1558
3. Bassetti, M., Righi, E., Montravers, P., & Cornely, O. A. (2018). What has changed in the treatment of invasive candidiasis? A look at the past 10 years and ahead. *Journal of Antimicrobial Chemotherapy*, *73*(suppl_1), i14-i25. https://doi.org/10.1093/jac/dkx445
4. Berman, H. M., Westbrook, J., Feng, Z., Gilliland, G., Bhat, T. N., Weissig, H., ... & Bourne, P. E. (2000). The protein data bank. *Nucleic acids research*, *28*(1), 235-242. https://doi.org/10.1093/nar/28.1.235
5. Billings, M., Dye, B. A., Iafolla, T., Grisius, M., & Alevizos, I. (2017). Elucidating the role of hyposalivation and autoimmunity in oral candidiasis. *Oral diseases*, *23*(3), 387-394. **https://doi.org/10.1111/odi.12626**
6. Dibha, A. F., Wahyuningsih, S., Kharisma, V. D., Ansori, A. N. M., Widyananda, M. H., Parikesit, A. A., Rebezov, M., Matrosova, Y., Artyukhova, S., Kenijz, N., Kiseleva, M., Jakhmola, V., & Zainul, R. (2022). Biological activity of kencur (Kaempferia galanga L.) against SARS-CoV-2 main protease: In silico study. *International Journal of Health Sciences*, *6*(S1), 468–480. https://doi.org/10.53730/ijhs.v6nS1.4779
7. Doddanna, S. J., Patel, S., Sundarrao, M. A., & Veerabhadrappa, R. S. (2013). Antimicrobial activity of plant extracts on Candida albicans: An in vitro study. *Indian Journal of Dental Research*, *24*(4), 401.
8. Fox, E., & Nobile, C. (2013). The Role of Candida albicans Biofilms in Human Disease. Candida Albicans Symptoms Causes Treat.
9. Jaghoori, M. M., Bleijlevens, B., & Olabarriaga, S. D. (2016). 1001 Ways to run AutoDock Vina for virtual screening. *Journal of computer-aided molecular design*, *30*(3), 237-249. https://doi.org/10.1007/s10822-016-9900-9

10. Joseph, T. L., Namasivayam, V., Poongavanam, V., & Kannan, S. (2017). In silico approaches for drug discovery and development. *Frontiers in Computational Chemistry. Bentham Science Publishers*, *3*, 74. **Doi:** 10.2174/9781681081670117030003
11. Junqueira, J. C., Vilela, S. F., Rossoni, R. D., Barbosa, J. O., Costa, A. C. B., Rasteiro, V., ... & Jorge, A. O. C. (2012). Oral colonization by yeasts in HIV-positive patients in Brazil. *Revista do Instituto de Medicina Tropical de Sao Paulo*, *54*(1), 17-24. doi: 10.1590/S0036-46652012000100004
12. Lazaridis, T. (2002). Binding affinity and specificity from computational studies. *Current Organic Chemistry*, *6*(14), 1319-1332. **DOI:** https://doi.org/10.2174/1385272023373491
13. Lim, S. V., Rahman, M. B. A., & Tejo, B. A. (2011, December). Structure-based and ligand-based virtual screening of novel methyltransferase inhibitors of the dengue virus. In *BMC bioinformatics* (Vol. 12, No. 13, pp. 1-12). BioMed Central. https://doi.org/10.1186/1471-2105-12-S13-S24
14. Manik, A., & Bahl, R. (2017). A review on oral candidal infection. *Journal of Advanced Medical and Dental Sciences Research*, *5*(3), 54. http://jamdsr.com/uploadfiles/12ORALCANDIDALINFECTION54-57.20170422083202.pdf
15. Muadcheingka, T., & Tantivitayakul, P. (2015). Distribution of Candida albicans and non-albicans Candida species in oral candidiasis patients: Correlation between cell surface hydrophobicity and biofilm forming activities. *Archives of oral biology*, *60*(6), 894-901. https://doi.org/10.1016/j.archoralbio.2015.03.002
16. Nguyen, N. T., Nguyen, T. H., Pham, T. N. H., Huy, N. T., Bay, M. V., Pham, M. Q., ... & Ngo, S. T. (2019). Autodock vina adopts more accurate binding poses but autodock4 forms better binding affinity. *Journal of Chemical Information and Modeling*, *60*(1), 204-211. https://doi.org/10.1021/acs.jcim.9b00778
17. Patil, S., Rao, R. S., Majumdar, B., & Anil, S. (2015). Clinical appearance of oral Candida infection and therapeutic strategies. *Frontiers in microbiology*, 1391. https://doi.org/10.3389/fmicb.2015.01391
18. Scheraga, H. A., Khalili, M., & Liwo, A. (2007). Protein-folding dynamics: overview of molecular simulation techniques. *Annu. Rev. Phys. Chem.*, *58*, 57-83. DOI: 10.1146/annurev.physchem.58.032806.104614
19. Sethi, A., Joshi, K., Sasikala, K., & Alvala, M. (2019). Molecular docking in modern drug discovery: Principles and recent applications. *Drug discovery and development-new advances*, *2*, 1-21.
20. Shamsudin Khan, Y., Gutiérrez-de-Terán, H., Boukharta, L., & Åqvist, J. (2014). Toward an optimal docking and free energy calculation scheme in ligand design with application to COX-1 inhibitors. *Journal of chemical information and modeling*, *54*(5), 1488-1499. https://doi.org/10.1021/ci500151f
21. Sharma, N. K., & Jha, K. K. (2010). Molecular docking: an overview. *J. Adv. Sci. Res*, *1*(1), 67-72. DOI: 10.1016/j.arabjc.2011.10.007
22. Singh, A., Verma, R., Murari, A., & Agrawal, A. (2014). Oral candidiasis: An overview. *Journal of oral and maxillofacial pathology: JOMFP*, *18*(Suppl 1), S81. doi: 10.4103/0973-029X.141325
23. Singh, Y., Karicheri, R., & Nath, D. (2022). The burden of catheter associated urinary tract infection by candida Albicans and non Albicans with emphasis

on biofilm formation and antifungal sensitivity pattern. *International Journal of Health Sciences*, *6*(S2), 2356–2363. https://doi.org/10.53730/ijhs.v6nS2.5544

24. Srivastava, R., Tripathi, S., Unni, S., Hussain, A., Haque, S., Dasgupta, N., ... & Mishra, B. N. (2021). Silybin B and cianidanol inhibit Mpro and spike protein of SARS-CoV-2: Evidence from in silico molecular docking studies. *Current pharmaceutical design*, *27*(32), 3476-3489. https://doi.org/10.2174/1381612826666201210122726
25. Xiao, X., Min, J. L., Lin, W. Z., Liu, Z., Cheng, X., & Chou, K. C. (2015). iDrug-Target: predicting the interactions between drug compounds and target proteins in cellular networking via benchmark dataset optimization approach. *Journal of Biomolecular Structure and Dynamics*, *33*(10), 2221-2233. https://doi.org/10.1080/07391102.2014.998710
26. Yusufkhan, P. S., Deshmukh, S. R., & Farooqui, M. (2022). Design, synthesis, and biological evaluation of some methyl 2-(1H-pyrazol-4-ylthio)-1,2,3,4-tetrahydro-6-methylpyrimidine-5-carboxylate derivatives as potential DHFR inhibitors. *International Journal of Health Sciences*, *6*(S1), 1018–1040. https://doi.org/10.53730/ijhs.v6nS1.4853